Calcasieu Estuary Remedial Investigation/Feasibility Study (RI/FS): Baseline Ecological Risk Assessment (BERA)

Appendix C: Assessment of Risks to the Microbial Community in the Calcasieu Estuary

Prepared For:

CDM Federal Programs Corporation
8140 Walnut Hill Lane, Suite 1000
Dallas, Texas 75231

Under Contract To:

Mr. John Meyer, Regional Project Manager
U.S. Environmental Protection Agency, Region 6
1445 Ross Avenue
Dallas, Texas 75202

Prepared – October 2002 – By:

MacDonald Environmental Sciences Ltd.
#24 - 4800 Island Highway North
Nanaimo, British Columbia V9T 1W6

In Association With:

United States Geological Survey
4200 New Haven Road
Columbia, Missouri 65201

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Appendix C. Assessment of Risks to the Microbial Community in the Calcasieu Estuary

1.0 Introduction

In response to concerns regarding environmental contamination in the Calcasieu Estuary, a Remedial Investigation/Feasibility Study (RI/FS) is being conducted in the estuary. One of the objectives of the RI/FS is to assess the risks posed by environmental contamination to ecological receptors that inhabit key areas of the Calcasieu Estuary. To meet this objective, a baseline ecological risk assessment (BERA) must be undertaken in accordance with the procedures laid out by the United States Environmental Protection Agency (USEPA) in the *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments* (USEPA 1997a). Under the eight-step process described by the USEPA for conducting a BERA, a screening ecological risk assessment (SERA) must be conducted to provide preliminary estimates of exposure and risk.

In 1999, CDM Federal Programs Corporation (CDM) conducted a screening level ecological risk assessment (SERA) for the Calcasieu Estuary which concluded that there was a potential risk to ecological receptors inhabiting the estuary from exposure to contaminated sediment and/or surface water (CDM 1999). In September 2001, a Baseline Problem Formulation (BPF; Appendix A; MacDonald *et al.* 2001) was prepared that identified chemicals of potential concern (COPCs) and areas of interest, described the environmental fate and ecological effects of the COPCs, and identified key exposure pathways and receptors at risk in the Estuary. The BPF also led to the development of assessment and measurement endpoints, a conceptual model and a risk analysis plan for the BERA. Accordingly, the BPF defined the issues that needed to be addressed in the BERA for the Calcasieu Estuary.

One of the important conclusions of the BPF was that microbial communities are likely to be exposed to various COPCs that occur in whole sediments, surface water, and pore water, with contact with whole sediment representing the most important and direct route of exposure. The other groups of aquatic receptors that are addressed in the Calcasieu Estuary BERA include: aquatic plant communities (Appendix D); benthic invertebrate communities (Appendix E2); fish communities (Appendix F1 and F2); avian communities (Appendix H); and, mammalian communities (Appendix I). The COPCs in whole sediments, surface water, and/or pore water that were identified in the BPF included various metals (i.e., copper, chromium, lead, mercury, nickel, and zinc), chlorinated ethanes (i.e., 1,2-dichloroethane and trichloroethane; DCE and TCA), polycyclic aromatic hydrocarbons (PAHs; i.e., 13 parent PAHs and total PAHs), polychlorinated biphenyls (PCBs; i.e., various Aroclor mixtures, PCB congeners, and total PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans [PCDFs; i.e., expressed as tetrachlorodibenzo-*p*-dioxins - toxic equivalents (TCDD-TEQs)], hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), bis(2-ethylhexyl)phthalate (BEHP), carbon disulfide, unionized ammonia, hydrogen sulfide, acetone, and several organochlorine pesticides (i.e., aldrin and dieldrin; see Table A1-7 of Appendix A1).

1.1 Conceptual Model

The conceptual site model represents a particularly important component of the problem formulation process because it enhances the level of understanding regarding the relationships between human activities and ecological receptors at the site under consideration. Specifically, the conceptual site model describes key relationships between stressors and assessment endpoints. In so doing, the conceptual model provides a framework for predicting effects on ecological receptors and a template for generating risk questions and testable hypotheses (USEPA 1997a; 1998). The conceptual site model also provides a means of highlighting what is known and what is not known about a site. In this way, the conceptual model provides a basis for identifying data gaps and designing monitoring programs to acquire the information necessary to complete the assessment. Conceptual site models consist of two main elements:

- A set of hypotheses that describe predicted relationships between stressors, exposures, and assessment endpoint responses (along with a rationale for their selection); and,
- Diagrams that illustrate the relationships presented in the risk hypotheses.

The conceptual site model of the Calcasieu Estuary is described in Chapter 7 of the BPF (Appendix A). More specifically, that chapter summarizes the available information on the sources and releases of COPCs, the fate and transport of these substances, the pathways by which ecological receptors are exposed to the COPCs, and the potential effects of these substances on the ecological receptors that occur in the Calcasieu Estuary. In turn, this information was used to develop a series of hypotheses that provide predictions regarding how ecological receptors are exposed to and respond to the COPCs. The conceptual site model, which describes the exposure pathways of greatest interest for microbial communities, was adopted for use in this deterministic risk assessment. Based on the pathways identified in the conceptual model, whole sediment is likely to represent the most important route of exposure for the microbial community (MacDonald *et al.* 2000a). For this reason, other possible exposure pathways (e.g., surface water and pore water) were not evaluated relative to the potential for adverse effects on the microbial communities in the estuary. Because the exposure estimates and chronic toxicity benchmarks are the same for microbes as they are for benthic invertebrates and fish, the risks to the microbial community associated with exposure to surface water and/or pore water would be comparable to those estimated for benthic invertebrates (Appendix E2) and fish (Appendix F1).

1.2 Areas of Concern

The Calcasieu River is one of the largest river systems in southwest Louisiana (LA). From its headwaters in the vicinity of Kisatchie National Forest (in Vernon Parish), the Calcasieu River flows some 260 km to the Gulf of Mexico near Cameron, LA (Figure C-1). While much of the Calcasieu River system is relatively uncontaminated, the portion of the watershed from the saltwater barrier near Lake Charles, LA to the Intercoastal Waterway has undergone extensive industrial development over the past five decades. These developmental activities have resulted in widespread contamination in the estuarine portion of the watershed, particularly in the bayous within the upper portion of the estuary (Curry *et al.* 1997).

In response to public concerns, USEPA is conducting a federally-led RI/FS to assess risks to human health and ecological receptors and to evaluate remedial options for addressing environmental contamination in the Calcasieu Estuary. Based on the results of the SERA, the portion of the Calcasieu Estuary from the saltwater barrier to Moss Lake was identified as the area in which environmental contamination posed the greatest potential risks to ecological receptors and, as such, was designated as the primary study area (CDM 1999). To facilitate the RI/FS, this study area was divided into four sub-areas (termed Areas of Concern; AOCs), including:

- Upper Calcasieu River AOC (UCR AOC);
- Bayou Verdine AOC (BV AOC);
- Bayou d'Inde AOC (BI AOC); and,
- Middle Calcasieu River AOC (MCR AOC).

Several reference areas were also identified in the lower estuary and in the vicinity of Sabine National Wildlife Refuge to support the interpretation of the data generated during the RI. As a BERA of Bayou Verdine has already been completed by Conoco, Inc. and Condea Vista (Entrix, Inc. 2001), ecological risks in the BV AOC were not assessed in this report.

1.3 Chemicals of Potential Concern

The identification of COPCs represents an essential element of the problem formulation process (USEPA 1998). To initiate this process, CDM conducted a SERA of the Calcasieu Estuary in 1999 to assess the potential for adverse biological effects on ecological receptors associated with either direct or indirect exposure to contaminated environmental media in the Calcasieu Estuary (CDM 1999). To support this assessment, historical data on the levels of environmental contaminants in surface water, sediment, and biota were collated and compiled (CDM 1999). Subsequently, the maximum measured concentration of each substance in each media type was compared to the lowest ecological screening value for that substance to facilitate the determination of maximum hazard quotients. These maximum hazard quotients provided a basis for identifying the substances in surface water, sediment, and biota of the estuary that occurred at levels sufficient to potentially adversely affect one or more ecological receptors. These substances were termed COPCs in the Calcasieu Estuary and included: metals; PAHs; PCBs (polychlorinated biphenyls);

organochlorine and other pesticides; chlorophenols; chlorinated benzenes; chlorinated ethanes; phthalates; cyanide; and, acetone.

Because the preliminary list of COPCs that emerged from the SERA contained over 100 substances (CDM 1999), it was determined that it required further refinement to assure that only those substances with a relatively high probability of adversely affecting ecological receptors were addressed in further investigations. For this reason, a scoping meeting was convened in Denver, Colorado (CO) in July, 2000 to develop a more focused list of COPCs. The scoping meeting was attended by risk assessors, risk managers, and the USEPA Region VI Ecological Technical Assistance Group (ETAG). Rather than relying on historical data (as was done in the SERA), the participants at this scoping meeting used the results of the Phase I sampling program of the RI to identify the COPCs in the Calcasieu Estuary (Goldberg 2001). For water-borne contaminants, the substances that occurred in unfiltered water samples at total concentrations in excess of the ambient water quality criteria (WQC; i.e., criteria continuous concentrations; CCCs; USEPA 1999) were deemed to be COPCs. For sediment-associated constituents, the substances that occurred in whole sediments at concentrations in excess of the effects range median values (ERMs; Long *et al.* 1995) or comparable sediment quality benchmarks (e.g., probable effect levels PEL; MacDonald *et al.* 1996; CCME 1999) were considered to be COPCs. Based on the results of these evaluations, the scoping meeting participants agreed that the following substances were the primary COPCs in the Calcasieu Estuary:

Water-Borne COPCs

- Metals (copper and mercury];
- 1,2-dichloroethane; and,
- Trichloroethane.

Sediment-Associated COPCs

- Metals (copper, chromium, lead, mercury, nickel, and zinc);
- Polycyclic aromatic hydrocarbons (PAHs; acenaphthene, acenaphthylene, anthracene, fluorene, 2-methylnaphthalene, naphthalene, phenanthrene, benz(a)anthracene, benzo(a)pyrene, chrysene, dibenz(a,h)anthracene, fluoranthene, pyrene, total PAHs, and other PAHs);
- Polychlorinated biphenyls (PCBs);
- Polychlorinated dibenzo-*p*-dioxins (PCDDs), and, polychlorinated dibenzofurans (PCDFs);
- Chlorinated benzenes [hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), and degradation products];
- Phthalates [bis(2-ethylhexyl)phthalate (BEHP)];
- Carbon disulfide;
- Unionized ammonia;
- Hydrogen sulfide;
- Acetone; and,
- Organochlorine pesticides (aldrin and dieldrin).

Because exposure to whole sediments represents the principal route through which the microbial community can be exposed to toxic chemicals, the substances that tend to become associated with sediment were identified as COPCs relative to the microbial community.

1.4 Purpose of Appendix

The purpose of this appendix is to provide an evaluation of the risks posed to the microbial community associated with exposure to COPCs in the Calcasieu Estuary. Microbial communities, which consist of bacteria, protozoans, and fungi, play several essential roles in estuarine ecosystems. Estuaries, in general, and salt marshes, in particular, are widely recognized as highly productive ecosystems (Odum 1975). While phytoplankton (i.e., the algae that is suspended in the water column) and periphyton (i.e., the algae that are attached to the bottom, to plants, or to animals) represent important primary producers (i.e., organisms that transform the sun's energy into organic material) in aquatic ecosystems, marsh grasses (such as *Spartina* sp.) are among the most important in salt marshes. Unlike algae, however, the emergent marsh plants often cannot be grazed directly because their tissues can be indigestible to higher order consumers (e.g., benthic macroinvertebrates; Apple *et al.* 2001). Consequently, this important source of energy can be utilized more effectively by higher-order consumers after it has been transformed by the microbial community. As such, the microbial community represents an important food source for shrimp, small crabs, worms, shellfish, and snails (Apple *et al.* 2001).

In addition to degrading and transforming detrital organic matter, microbial communities also play a number of key roles in the cycling and transformation of nutrients in sediments and the water column (Odum 1975). For example, the microbial community is an essential component of the nitrogen cycle, in which atmospheric nitrogen is converted, through a series of steps, into nitrates, nitrites, and ammonia. These forms of nitrogen represent essential plant nutrients and are the basic building blocks for protein synthesis (Colinvaux 1973). The sulfur cycle in aquatic environments, in which hydrogen sulfide is converted to sulfate (which is incorporated into plant and animal tissues), is also mediated by the microbial community (Odum 1975). Furthermore, the microbial community supports primary productivity by transforming phosphorus into forms that can be readily used by aquatic plants (i.e., phosphate). Finally, carbon cycling (i.e., between the dissolved and particulate forms) in aquatic ecosystems is dependent on the microbial community. Although specific information on the composition of microbial communities in the Calcasieu Estuary was not located, it is certain that the microbial community plays an essential ecological role in this watershed.

To date, the ecological assessment work conducted for the Calcasieu Estuary has not characterized the potential risks to the microbial community associated with exposure to COPCs in environmental media. Risk hypotheses laid out in the BPF indicate that many COPCs pose a potential risk to the microbial community from direct contact with contaminated sediments and/or pore water. Subsequently, discussions among the members of the ETAG indicated that pore water and surface water are likely to represent less important exposure routes for the microbial community. As such, whole sediment was identified as the principal route of exposure to COPCs for the microbial community. This appendix provides an evaluation of the risks posed to the microbial community associated with exposure to the COPCs in the Calcasieu Estuary.

2.0 Methods

A step-wise approach was used to assess the risks to the microbial community posed by exposure to COPCs in the Calcasieu Estuary. The five main steps in this process included:

- Identification of assessment endpoints, risk questions and testable hypotheses, and measurement endpoints;
- Collection, evaluation, and compilation of the relevant information on sediment quality conditions in the Calcasieu Estuary;
- Assessment of the exposure of the microbial community to COPCs (i.e., exposure assessment);
- Assessment of the effects of COPCs on the microbial community (i.e., effects assessment); and,
- Characterization of risks to the microbial community (i.e., risk characterization).

Each of these steps is described in the following sections of this appendix.

2.1 Identification of Assessment and Measurement Endpoints

An assessment endpoint is an 'explicit expression of the environmental value that is to be protected' (USEPA 1997a). The selection of assessment endpoints is an essential element of the overall ecological risk assessment (ERA) process because it provides a means of focusing assessment activities on the key environmental values (e.g., reproduction of sediment-probing birds) that could be adversely affected by exposure to environmental contaminants. Assessment endpoints must be selected based on the ecosystems, communities, and species that occur, have historically occurred, or could potentially occur at the site (USEPA 1997a).

A measurement endpoint is defined as 'a measurable ecological characteristic that is related to the valued characteristic that is selected as the assessment endpoint' and it is a measure of biological effects (e.g., mortality, reproduction, growth; USEPA

1997a). Measurement endpoints are frequently numerical expressions of observations (e.g., toxicity test results, community diversity measures) that can be compared to similar observations at a control and/or reference site. Such statistical comparisons provide a basis for evaluating the effects that are associated with exposure to a contaminant, or group of contaminants, at the site under consideration.

To support the identification of key assessment and measurement endpoints for the Calcasieu Estuary BERA, the USEPA convened a BERA workshop in Lake Charles, LA on September 6 and 7, 2000. The workshop participants included representatives of the USEPA, United States Geological Service (USGS), National Oceanic and Atmospheric Administration (NOAA), Louisiana Department of Environmental Quality (LDEQ), United States Fish and Wildlife Service (USFWS) and CDM (hereafter termed the Calcasieu Estuary Ecological Risk Assessment Advisory Group). The workshop was explicitly designed to enable participants to articulate the goals and objectives for the ecosystem (i.e., based on the input that had been provided by the community in a series of public meetings), to assess the status of the knowledge base, to clearly define key issues and concerns, and to identify the COPCs and AOCs in the study area. Importantly, this workshop approach provided a basis for refining the candidate assessment endpoints that had been proposed based on the results of the SERA (CDM 1999). Additionally, workshop participants identified a suite of measurement endpoints that would provide the information needed for evaluating the status of the assessment endpoints (MacDonald *et al.* 2000a).

To be effective, the measurement endpoints must be linked to the assessment endpoints by a series of risk questions. These risk questions, which can be restated as testable hypotheses, describe the specific assumptions about the potential risk to assessment endpoints posed by exposure to COPCs in environmental media. The risk questions were developed using a combination of professional judgement and information on the potential sources of stressors, stressor characteristics, and actual and predicted ecological effects on the selected assessment endpoints (USEPA 1998). The conceptual model diagrams presented in the BPF provide a visual representation of the risk hypotheses.

2.2 Collection, Evaluation, and Compilation of Relevant Information on Sediment Quality Conditions in the Calcasieu Estuary

Information on the chemical and toxicological characteristics of whole sediments were collected in two phases, including Phase I and Phase II sampling programs. The methods that were used to collect the samples, quantify the levels of COPCs in various media types, evaluate the toxicity of sediment samples, evaluate the reliability of the resultant data, and compile the information in a form that would support the BERA are described in the following sections.

Sample Collection - The RI of the Calcasieu Estuary was conducted in two phases. In Phase I of the investigation, more than 500 sediment samples were collected at sites located throughout the estuary between November, 1999 and March, 2000, based on a stratified random sampling design. The samples collected during this phase of the sampling program were intended to provide the data needed to assess the nature and extent of contamination within the estuary. The Phase II sampling program was designed to augment the data that were collected in Phase I by providing further information on the nature, severity and areal extent of contamination, assessing the bioavailability of environmental contaminants, evaluating the effects on ecological receptors associated with exposure to contaminants, and filling outstanding data gaps. In Phase II of the investigation, more than 100 sediment samples were obtained, again based on a stratified random sampling design. The locations of the sampling sites in the UCR and BV AOCs, BI AOC, MCR AOC, and reference areas are shown in Figures C-2, C-3, C-4a, C-4b, and C-5, respectively.

As indicated above, a stratified random sampling design was utilized in both phases of the RI. More specifically, the estuary was divided into five areas (i.e., UCR AOC, BV AOC, BI AOC, MCR AOC, and reference areas), multiple reaches within each area, and numerous sub-reaches within each reach. Subsequently, the number of samples that were to be collected within each area, reach, and sub-reach was determined based on the size of the area, historic contamination patterns, and other factors. Then, the USEPA Region V Fully Integrated Environmental Location Decision Support (FIELDS) tools were used to randomly select coordinates (i.e., latitude and longitude) for the assigned number of primary sampling stations and alternate sampling stations (i.e., which would be sampled if it was not possible to obtain samples from any primary sampling stations). In the field, each sampling station was located with the aid of navigation charts and a Trimble differentially-corrected global positioning system (GPS). Sediment samples were collected within a 10 meter radius of the designated sampling coordinates.

The methods that were used to collect, handle, and transport the sediment samples collected in the Phase I and Phase II sampling programs are described in CDM (2000a; 2000b; 2000c; 2000d; 2000e). Briefly, surficial sediment samples (i.e., the top 10 cm) were collected with a modified large Eckman dredge (23 x 23 cm). Deeper sediment samples were collected with a piston sampler. At each station, multiple grab samples (up to 10 grabs) were obtained, composited, and homogenized to support the preparation of splits for various chemical analyses, pore-water extraction, and/or toxicity tests. All whole-sediment samples were shipped to laboratories in plastic coolers on ice.

Chemical Analyses - Chemical analysis of the sediment samples collected during the Phase I and Phase II sampling programs was conducted at various contract laboratory program (CLP) and subcontract (non-CLP) analytical laboratories, including Quanterra-Severn Trent Laboratories, USEPA Region VI Laboratory, Texas A&M University laboratories, USEPA Region VI CLP laboratories, Olin Contract laboratories, and PPG Industries contract laboratories. Upon receipt at the laboratory, sediment and pore-water samples were held in coolers until selection for analysis.

In Phase I, total metals in whole sediments were quantified using a variety of analytical methods, including E6020, SW6010B, SW7471A, and SW6020. Polycyclic aromatic hydrocarbons and/or other semi-volatile organic compounds (SVOCs) were quantified using one or more of the following methods, SW8260B, SW8270C, HOU-SVOC, SW8015B, and SW8015B MOD. Method SW8260B was used to quantify volatile organic compounds. Polychlorinated biphenyls were quantified using SW8081A, while pesticides and herbicides were quantified using SW8081A and SW8151A, respectively. Finally, PCDDs and PCDFs were measured using SW8290. A summary of the analyses that were conducted at each analytical laboratory is presented in Table C-1.

In Phase II, total and total recoverable metals were analyzed in whole sediments by the USGS Columbia Environmental Research Center using inductively-coupled plasma-mass spectrometry (ICP-MS; May *et al.* 1997). Methods SW6010B, SW7471A, 200.7, and 200.9 were used by American Analytical and Technical Services (AATS) and USEPA Region VI Laboratory to quantify the concentrations of total recoverable metals in whole-sediment samples. Total and methyl mercury were analyzed by Texas A&M University using methods SW7470A, SW7471B, and USGS 005. Polycyclic aromatic hydrocarbons and other semi-volatile organic compounds were measured by AATS and USEPA Region VI Laboratory using SW 8270C, while PCBs Aroclors were measured by AATS and USEPA Region VI Laboratory using SW8082. Polychlorinated biphenyl congeners were analyzed by ALTA Laboratories using SW1668, while polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and PCDDs were analyzed by ALTA Laboratories using SW8290 (Table C-1).

Whole-Sediment Toxicity - The Microtox® Solid-Phase Toxicity (SPT) test was conducted on 100 whole-sediment samples collected during the Phase II sampling program (MESL 2001). In the SPT test, bioluminescent bacteria (*Vibrio fisheri*; B-NRL 1117, Azur Environmental, Carlsbad, California) were exposed directly to sediment suspended in solution for a period of 20 minutes at 15°C in a temperature controlled water-bath (Microbics Corporation 1992; Johnson 1998; Johnson and Long 1998; Ingersoll *et al.* 2001). A log-linear model was used to calculate median effective concentration (EC_{50}) values (expressed as percentage of sediment wet weight/mL; Johnson and Long 1998) and 95% confidence intervals for each sediment tested.

Sediment samples were designated as toxic or not toxic using a reference envelope approach. In this approach, the statistical distribution of the EC_{50} values for the samples from the reference areas was first determined. These values were found to be lognormally distributed. Next, the normal range of the EC_{50} values for the reference samples (i.e., 95% prediction limits) was determined by calculating the 2.5^{th} and 97.5^{th} percentile values. Samples were designated as toxic if the calculated EC_{50} values were lower than the lower limit of the normal range of EC_{50} values (i.e., the 2.5th percentile value; if the EC_{50} was less than 1.4% sediment wet weight/mL). All other samples were designated as not toxic.

Although several other procedures could have been used to designate samples as toxic or not toxic [e.g., analysis of variance (ANOVA) compared to control, paired T-tests with control results, minimum significant difference from control; Thursby *et al.* 1997], the reference envelope approach was utilized because it provides a means of evaluating incremental toxicity at test sites when compared to reference sites (Hunt *et al.* 2001). In this way, only the toxicity attributable to differences in the characteristics of test and reference samples was considered for the purposes of the BERA. That is, the reference envelope approach provides a basis of determining the toxicity that is attributable primarily to COPC-related factors in the estuary.

Data Validation and Verification - All of the data sets that were generated during the course of the study were critically reviewed to determine their applicability to the assessment of risks to the microbial community in the Calcasieu Estuary. The first step in this process involved validation of all of the sediment chemistry data. Following translation of these data and the sediment toxicity data into database format, the validated data were then further evaluated to assure the quality of the data used in the risk assessment. This auditing process involved analyses of outliers (i.e., to identify inconsistencies with units) and completeness (i.e., to identify missing samples or missing data); examination of data qualifier fields (i.e., to assure internal consistency in the BERA database); and checking of sample identification numbers (to ensure that data were not duplicated). Finally, the data were fully verified against the original data source.

Database Development - To support the compilation and subsequent analysis of the information on sediment quality conditions in the Calcasieu Estuary, a relational project database was developed in Microsoft Access format. All of the sediment chemistry and sediment toxicity data compiled in the database were georeferenced to facilitate mapping and spatial analysis using geographic information system (GIS)-based applications [i.e., Environmental Systems Research Institute, Inc (ESRI's) ArcView and Spatial Analyst programs]. The database structure made it possible to retrieve data in several ways, including by data type (i.e., chemistry vs. toxicity), by sediment horizon (i.e., surficial vs. sub-surface sediments), by stream reach (e.g., Upper Bayou d'Inde), by sub-reach (i.e., Upper Bayou d'Inde-1 vs. Upper Bayou d'Inde-2), and by date (i.e., Phase I vs. Phase II). As such, the database facilitated a variety of data analyses.

2.3 Assessment of Exposure of Microorganisms to Chemicals of Potential Concern

To facilitate assessment of risks to the microbial community, the sediment chemistry data that were collected during the Phase I and Phase II sampling programs were summarized. More specifically, summary statistics were calculated for each reach and AOC of the study area, including the number of samples collected (n), mean and standard deviation, 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles, geometric mean, and range for each COPCs. The mean, standard deviation, and percentile statistics were calculated on $\log_e$ transformed data, based on the assumption that the underlying data were log-normally distributed. Because previous studies have demonstrated that normalization of contaminant concentrations to the variables that are thought to influence bioavailability [e.g., total organic carbon (TOC), grain size, acid volatile sulfides (AVS); Barrick *et al.* 1988, Ingersoll *et al.* 1996; Long *et al.* 1998] does not improve classification of field-collected sediment samples as toxic or not toxic, contaminant concentrations were expressed on a dry-weight basis.

2.4 Assessment of the Effects of Chemicals of Potential Concern on Microorganisms

In this assessment, exposure of the microbial community to COPCs was evaluated using information on the concentrations of contaminants in whole sediments. As such, it was necessary to compile information on the effects on microbial communities associated with exposure to COPCs in these environmental media. Evaluation of the potential effects of sediment-associated contaminants on microbial communities necessitated the selection of toxicity thresholds for whole sediments (i.e., sediment quality benchmarks).

Numerical benchmarks (including sediment quality criteria, sediment quality objectives, and sediment quality standards) represent useful tools for assessing the quality of freshwater and marine sediments (USEPA 1992; Adams *et al.* 1992; USEPA 1996; 1997b; Ingersoll and MacDonald 1999; MacDonald *et al.* 2000b; 2000c). Such benchmarks have been developed by various jurisdictions in North America using a variety of approaches. The approaches that have been selected by individual jurisdictions depend on the receptors that are to be considered (e.g., sediment-dwelling organisms, wildlife, or humans), the degree of protection that is to be afforded, the geographic area to which the values are intended to apply (e.g., site-specific, regional, or national), and their intended uses (e.g., screening tools or remediation objectives). The sediment quality guidelines (SQGs) that have been promulgated by various jurisdictions throughout North America were reviewed and evaluated to identify relevant benchmarks for assessing sediment quality conditions in the Calcasieu Estuary relative to effects on the microbial community (Table C-2). In addition, an evaluation of the predictive ability of numerical SQGs is provided in Appendix E1.

2.5 Characterization of Risks to the Microbial Community

The characterization of the risks to the microbial community consisted of three main steps. First, the nature, severity, and areal extent of risks to the microbial community were evaluated using one or more lines of evidence. Next, the substances that are causing or substantially contributing to effects on the microbial community were identified [i.e., contaminants of concern (COCs)]. Finally, the information on multiple lines of evidence was integrated to evaluate the risks to the microbial community associated with exposure to COCs. The methods that were used in each of these steps of the process are described in the following sections.

Evaluation of the Nature, Severity, and Areal Extent of Risks - In this assessment, data on two measurement endpoints were used to evaluate the risks posed by COPCs to the microbial community. These lines of evidence included whole-sediment chemistry and whole-sediment toxicity. More specifically, the results of whole-sediment toxicity tests were used to evaluate the nature of the risks to the microbial community. Both lines of evidence were used to evaluate the magnitude (i.e., severity) of risks to the microbial community. Finally, whole-sediment chemistry data were used to conduct a preliminary evaluation of the areal extent of risks to the microbial community.

To facilitate characterization of the magnitude and areal extent of risks to the microbial community, risks were classified into three categories for each sample, reach, and AOC. More specifically, risks to the microbial community were characterized as low, indeterminate, or high, based on the observed incidence of sediment toxicity, the observed magnitude of sediment toxicity, and the predicted incidence of sediment toxicity. The following criteria for classifying risks were established based on the guidance that was provided by the Calcasieu Estuary Ecological Risk Assessment Advisory Group (MacDonald *et al.* 2000a; 2001; Table C-3).

Low Risks - ecological risks were classified as low if the effects that were observed or predicted to occur within a sample, reach, or an AOC were similar in frequency and/or magnitude to those for selected references areas (Table C-3). Such effects were considered to be negligible relative to the maintenance of the structure and/or function of the microbial community within a reach or an AOC. Nevertheless, conditions that require attention may exist within portions of a reach or AOC that was classified as having low risks to the microbial community. Low risks were indicated by:

- 0 to <20% increase in the observed incidence of toxicity (% toxic samples) to marine bacteria within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 25-minute SPT with the bacterium, *Vibrio fisheri;* endpoint: EC_{50}-bioluminescence).

- 0 to <20% increase in the predicted incidence of toxicity to bacteria within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on whole-sediment chemistry and the Microtox apparent effects thresholds (AETs); samples with one or more exceedances of the AETs were predicted to be toxic).

- 0 to <10% increase in the observed magnitude of toxicity (i.e., EC_{50}) to marine bacteria within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 25-minute SPT with the bacterium, *Vibrio fisheri*; endpoint: EC_{50}-bioluminescence).

Indeterminate Risks - ecological risks were classified as indeterminate if the effects that were observed or predicted to occur within a sample, a reach, or an AOC were moderately higher in frequency and/or magnitude than those for selected references areas (Table C-3). Such effects were considered to be of concern relative to the maintenance of the function of the microbial community within a reach or an AOC. Although such risks are nontrivial, decisions regarding remediation at individual locations should consider the costs and ecological effects of remedial actions, the potential for natural restoration, and other relevant factors. It is important to note that low or high risks to the microbial community could exist within portions of a reach or AOC that was classified as posing indeterminate risks. Indeterminate risks were indicated by:

- 20 to 50% increase in the observed incidence of toxicity (i.e., % toxic samples) to marine bacteria within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 25-minute SPT with the bacterium, *Vibrio fisheri*; endpoint: EC_{50}-bioluminescence).

- 20 to 50% increase in the predicted incidence of toxicity to bacteria within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on whole-sediment chemistry and the

Microtox AETs; samples with one or more exceedances of the AETs were predicted to be toxic).

- 10 to 20% increase in the observed magnitude of toxicity to marine bacteria within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 25-minute SPT with the bacterium, *Vibrio fisheri*; endpoint: EC_{50}-bioluminescence).

High Risks - ecological risks were classified as high if the effects that were observed or predicted to occur within a sample, a reach, or an AOC were substantially higher in frequency and/or magnitude than those for selected references areas (Table C-3). Such effects were considered to be the highest concern relative to the maintenance of the function of the microbial community within a reach or an AOC. Reaches or AOCs so designated represent the highest priority areas for remedial action planning. It is important to note that low or indeterminate risks to the microbial community could exist within portions of a reach or AOC that was classified as posing high risks. Therefore, any remedial actions that are contemplated within such reaches or AOCs should consider the severity and areal extent of the observed and predicted effects. High risks were indicated by:

- >50% increase in the observed incidence of toxicity (i.e., % toxic samples) to marine bacteria within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 25-minute SPT with the bacterium, *Vibrio fisheri*; endpoint: EC_{50}-bioluminescence).

- >50% increase in the predicted incidence of toxicity to bacteria within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on whole-sediment chemistry and the Microtox AETs; samples with one or more exceedances of the AETs were predicted to be toxic).

- >20% increase in the observed magnitude of toxicity (i.e., EC_{50}) to marine bacteria (based on % survival) within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 25-minute SPT with the bacterium, *Vibrio fisheri*; endpoint: EC_{50}-bioluminescence).

Identification of Contaminants of Concern - The COCs in the Calcasieu Estuary were identified using a step-wise approach. In the first step of this process, the concentrations of COPCs in whole sediments in each reach of the estuary (i.e., 95th percentile concentrations) were compared to the concentrations of COPCs in whole sediments from the reference areas (i.e., 95th percentile concentrations; the upper limit of background levels). The substances that occurred in the areas of concern at concentrations that were a factor of two or greater than the upper limit of background concentrations (i.e., 95th percentile concentrations) in the reference areas were retained as preliminary COCs in whole-sediments. Substances were also retained for further assessment if the 95th percentile concentration could not be calculated for the reference area, or if the 95th percentile concentration could not be calculated for one or more reaches in an AOC. In both cases, the substance was designated as an uncertain COC. The substances that were designated as preliminary or uncertain COCs were considered to pose potential incremental risks to the microbial community.

In the second step of the process, the estimates of the upper limit of the concentrations of preliminary COCs in whole sediments (i.e., 95th percentile concentrations) in each reach of the study area were compared to the corresponding chemical benchmark (Table C-2). Substances for which the 95th percentile concentration in whole-sediment samples in one or more reaches exceeded the selected benchmark were retained as preliminary COCs relative to the microbial community (i.e., the substances for which hazard quotients (HQ) of > 1 were calculated, where HQ = concentration ÷ benchmark). A substance was designated as an uncertain COC if there was no benchmark available for the substance or if the 95th percentile concentration could not be determined for one or more reaches within an AOC (i.e., due to high detection limits).

In the final step of the process, cumulative concentration distribution functions were generated for selected preliminary and uncertain COCs identified in Step 1 and 2 above, using the sediment chemistry data collected in Phase II. More specifically, the matching sediment toxicity and sediment chemistry data were used to develop estuary-wide distribution functions for both toxic and non-toxic sediment samples (i.e., based on the results of the whole-sediment toxicity). Substances were retained as COCs if the cumulative distribution functions for the toxic and non-toxic samples diverged substantially in the upper portion of the concentration range (i.e., the 75th percentile concentration for the effects distribution was a factor of two or more greater than the 75th percentile concentration for the no effects distribution; Long *et al.* 1995)

Integrated Assessment of the Risks to the Microbial Community using a Weight of Evidence Approach - In this assessment, data from chemical analyses, and toxicity tests were used to characterize risks to the microbial community associated with exposure to COPCs in the Calcasieu Estuary. More specifically, the data of up to two lines of evidence, generated during the RI, were used together to estimate risks to the microbial community exposed to whole sediments in the study area. The first step in this process was to calculate a risk score for each measurement endpoint and each line of evidence. Each measurement endpoint was examined to determine if low, indeterminate or high risks were indicated for each sample. A raw risk score of 0, 1, or 2, was assigned to the measurement endpoint based on the risk classification that was assigned (i.e., low, indeterminate, or high, respectively). Next a total evaluation score (TES; i.e., between 1 - low and 3 - high) was calculated to determine the weight that should be placed on the resultant data. The TES was determined by considering a variety of important attributes of the measurement endpoint. By multiplying the magnitude of the risk (raw risk score) by the weight assigned to the measurement endpoint (i.e., as established by the TES), it was possible to calculate an endpoint risk score of between zero and six for each measurement endpoint. The risk scores for each measurement endpoint were then averaged to calculate an average risk score for each line of evidence for each sample. The information on multiple lines of evidence was then integrated using a simple arithmetic procedure. That is, the average risk score for the various lines of evidence were averaged to generate a final risk score for the assessment endpoint for each sample. Final risk scores of 0 - <2, 2 to 3, and >3 were considered to represent low, indeterminate, and high risks to the microbial community respectively.

3.0 Results and Discussion

The assessment of the risks to the microbial community posed by the exposure to the COPCs in the Calcasieu Estuary involved several steps. In the first step of the process, the assessment endpoints, risk questions and testable hypotheses, and measurement endpoints were identified (i.e., in the BPF). Next, the relevant information on sediment quality conditions in the Calcasieu Estuary were collected, evaluated, and compiled. Subsequently, the chemical benchmarks for assessing sediment quality conditions were selected. Finally, the risks to the microbial community associated with exposure to whole sediment from the Calcasieu Estuary were assessed. The results of these evaluations are presented in the following sections of this report.

3.1 Assessment Endpoints

Microbial communities, which consist of bacteria, protozoans, and fungi, play several essential roles in estuarine ecosystems. First, microbial communities transform the energy from aquatic organisms into forms that can be used directly by primary consumers, such as small crabs, worms, shellfish, and snails (e.g., by degrading and transforming detrital organic matter; Apple *et al.* 2001). Microbial communities also play a key role in the cycling and transformation of nutrients in sediments and the water column. For example, the microbial community is an essential element of the nitrogen cycle, in which atmospheric nitrogen is converted through a series of steps into nitrates, nitrites, and ammonia. These forms of nitrogen represent essential plant nutrients and are the basic building blocks for protein synthesis. The sulfur cycle in aquatic environments, in which hydrogen sulfide is converted to sulfate (which is incorporated into plant and animal tissues), is also mediated by the microbial community (Odum 1975). The microbial community supports primary productivity by transforming phosphorus into forms that can be readily used by aquatic plants (i.e., phosphate). Finally, carbon cycling (i.e., between the dissolved and particulate forms) in aquatic ecosystems is dependent on the microbial community.

As the microbial community supports a number of critical ecosystem functions (see above), it is important to evaluate the effects of environmental contaminants on this group of ecological receptors. Aquatic microorganisms, including bacteria, protozoans, and fungi, can be exposed to environmental contaminants through direct contact with contaminated surface water, through contact with contaminated sediments, and through contact with contaminated pore water. Of these pathways, exposure to contaminated sediments probably represents the primary route of exposure for epibenthic and infaunal microbial species. For this reason, it is important to evaluate the effects of exposure to contaminated sediments on the activity of the microbial community (i.e., the rate at which microorganisms perform essential ecosystem functions, such as processing organic carbon). As the goal of this assessment is to determine if contaminated sediments are likely to adversely affect the key functions that are provided by the microbial community, the **activity of the aquatic microbial community** was identified as the assessment endpoint for this component of the BERA.

3.2 Measurement Endpoints

It is difficult to measure the status of the assessment endpoint directly in the Calcasieu Estuary. For this reason, a suite of measurement endpoints were selected to provide the information needed to determine if the activity of the microbial community is being adversely affected due to exposure to COPCs. First, sediment chemistry data were used to determine if the concentrations of COPCs in Calcasieu Estuary sediments were sufficient to cause or substantially contribute to sediment toxicity. In addition, the results of solid-phase sediment toxicity tests with the bacterium, *Vibrio fisheri* [i.e., Microtox®; using the methods described in Johnson (1998) and in Johnson and Long (1998)] were used to evaluate the effects of contaminated sediments on the activity of the microbial community. More specifically, bioluminescence in the bacterium, *Vibrio fisheri,* was used as an indicator of microbial metabolic rate and, hence, the ability of the microbial community to perform key functions (such as carbon processing). Although *Vibrio fisheri* is a marine species, it has been used as a surrogate species for evaluating the effects of contaminants in surface water, pore water, sediments, and elutriates in freshwater and estuarine environments (Burton *et. al.* 1996; Johnson 1998; Johnson and Long 1998).

3.3 Risk Questions and Testable Hypotheses

To provide a valid basis for assessing ecological effects, the assessment endpoint needs to be linked to the measurement endpoints by a series of risk questions and testable hypotheses. In this study, the investigations to assess the effects of environmental contaminants on the microbial community were designed to answer the following risk questions:

- Are the levels of COPCs in whole sediment from the AOCs in the Calcasieu Estuary greater than the levels of COPCs in whole sediment from reference areas and greater than the sediment quality benchmarks for the protection of microorganisms that utilize benthic habitats (i.e., the activity of the microbial community)?

- Is the metabolic rate of bacteria (i.e., the activity of aquatic microbiota, as indicated by the bioluminescence of the bacterium, *Vibrio fisheri*) exposed to sediments from the Calcasieu Estuary AOCs outside the normal range (i.e., 95% Confidence interval) for bacteria exposed to reference sediments?

The linkages between the assessment endpoint and the measurement endpoints are articulated in greater detail in the BPF (Appendix A1).

3.4 Exposure of the Microbial Community to Chemicals of Potential Concern

Exposure is the contact or co-occurrence of a contaminant and a receptor (Suter *et al.* 2000). The exposure assessment is intended to provide an estimate of the magnitude of exposure of receptors to COPCs, over time and space. Both baseline exposure and potential future exposure need to be evaluated during the exposure analysis. For the microbial community, contaminated sediment was considered to be the principal route of exposure requiring analysis.

In this investigation, exposure of microbial community to COPCs was evaluated for three AOCs and 14 reaches within the study area. Because many of the COPCs considered in this assessment tend to be relatively persistent in sediments, the data that were collected during Phase I and Phase II of the RI were considered to be equivalent and used to assess both current and near-term future exposure to sediment-associated COPCs.

The data on the chemical composition of whole sediments in the Calcasieu Estuary that were collected during Phase I and Phase II of the RI are presented in Appendix B4. The data summaries for each reach include the number of samples collected (n), mean and standard deviation, geometric mean, 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, and 95^{th} percentiles, and range for each COPCs (See Tables C-4 to C-22). To facilitate comparisons among AOCs, these data were further summarized for each AOC (including the reference area; Tables C-23 to C-26). These AOC-specific data summaries include the number of samples in which the substance was detected, total number of samples for each chemical analyte, as well as the estimates of distributions and central tendency for each analyte. By comparing the concentrations of COPCs in each AOC to the upper limit of background concentrations (i.e., the 95% upper confidence limit (UCL) for sediment samples from reference areas), it is possible to identify the substances that occur at levels that could pose incremental risks to the microbial community, relative to the risk that they pose in reference sediments.

Upper Calcasieu River Area of Concern - Within the UCR AOC, the concentrations of chromium, copper, lead, mercury, methyl mercury, zinc, 1,1-biphenyl, numerous individual PAHs, total low molecular weight PAHs (LMW-PAHs), total high molecular weight PAHs (HMW-PAHs), total PAHs, various PCB congeners and PCB mixtures (Aroclors), total PCBs, BEHP, di-n-butylphthalate, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,1,1-trichloroethane, 1,2-dichloroethane, various PCDD/PCDFs, TCDD-TEQs, acetone, and carbon disulfide exceeded the 95% UCL for the reference areas (Tables C-23 and C-26). The concentrations of nickel, indeno(1,2,3-cd)pyrene, PCB 80, Aroclor 1221, dimethylphthalate, certain chlorinated benzenes, and various PCDDs, in UCR sediments were not elevated relative those in reference areas (Tables C-23 and C-26).

Bayou d'Inde Area of Concern - The results of Phase I and II of the RI indicate that certain COPCs occur in sediments from BI at levels that exceed the 95% UCL for reference sediments. More specifically, the concentrations of chromium, copper, lead, mercury, methyl mercury, nickel, zinc, 1,1-biphenyl, various individual PAHs, total LMW-PAHs, total HMW-PAHs, total PAHs, all of the PCB congeners and PCB mixtures (Aroclors), total PCBs, dieldrin, BEHP, di-n-butylphthalate, dimethylphthalate, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,1,1-trichloroethane, 1,2-dichloroethane, PCDDs/PCDFs, TCDD-TEQs, acetone, and carbon disulfide exceeded the 95% UCL for the reference areas (Tables C-24 and C-26).

Middle Calcasieu River Area of Concern - Elevated levels of various COPCs (relative to reference sediments) were measured in sediment samples from the MCR AOC. More specifically, the concentrations of chromium, copper, lead, mercury, methyl mercury, zinc, various individual PAHs, total LMW-PAHs, total HMW-PAHs, total PAHs, all of the PCB congeners and PCB mixtures (Aroclors), total PCBs, BEHP, di-n-butylphthalate, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,1,1-trichloroethane, 1,2-dichloroethane, PCDDs/PCDFs, TCDD-TEQs, acetone, and carbon disulfide exceeded the 95% UCL for the reference areas (Tables C-25 and C-26). The levels of sediment-associated nickel, 1,2,4-trichlorobenzene and 1,2-dichlorobenzene were similar in the MCR AOC and the reference areas (Tables C-25 and C-26).

Summary - The results of the exposure assessment indicate that a number of COPCs occur in whole-sediment samples from the Calcasieu Estuary at concentrations in excess of the 95% UCLs for the selected reference areas. These substances that occur in Calcasieu Estuary sediments at elevated levels relative to reference conditions include: chromium; copper; lead; mercury; methyl mercury;

nickel; zinc; 1,1-biphenyl; various individual PAHs; total LMW-PAHs; total HMW-PAHs; total PAHs; various PCB congeners and PCB mixtures (i.e., Aroclors); total PCBs; dieldrin; BEHP; di-n-butylphthalate; dimethylphthalate; 1,3-dichlorobenzene; 1,4-dichlorobenzene; 1,1,1-trichloroethane; 1,2-dichloroethane; PCDDs/PCDFs; TCDD-TEQs; acetone; and, carbon disulfide. As a result, it is concluded that these COPCs occur in whole sediments from Calcasieu Estuary at levels that could pose incremental risks to microbial communities.

3.5 Effects of Chemicals of Potential Concern on the Microbial Community

In the analysis of effects, risk assessors determine the nature of toxic effects that are associated with exposure to contaminants and their magnitude as a function of exposure (Suter *et al.* 2000). Information on the effects of environmental contaminants may be acquired from the results of single chemical toxicity tests (e.g., spiked-sediment toxicity tests), ambient media toxicity tests (e.g., the results of toxicity tests conducted using sediments collected from the site under investigation), and/or biological surveys (e.g., microbial community assessments). Importantly, the data that are collected during this phase of the assessment should be directly related to the exposure estimates (e.g., if the exposure estimates are based on dry weight concentrations of COPCs, the effects data should describe the responses of receptors to changing dry weight concentrations of that COPC), thereby facilitating characterization of risks to each assessment endpoint.

In this assessment, exposure of the microbial community to COPCs was evaluated using information on the concentrations of contaminants in whole sediments. As such, it was necessary to compile information on the effects on microbial communities associated with exposure to COPCs in these environmental media. For whole sediments, numerical sediment quality guidelines may be used to evaluate the effects on microorganisms associated with exposure to COPCs. Such SQGs can be developed using the results of equilibrium partitioning modeling, spiked-sediment toxicity tests, and/or investigations of *in situ* sediment quality conditions (i.e., whole-sediment toxicity tests and whole-sediment chemistry analyses; Ingersoll *et al.* 1997). To support the selection of toxicity thresholds for whole sediments, numerical SQGs were compiled from various sources for each of the COPCs identified in the BPF (i.e., Barrick *et al.* 1988; Long and Morgan 1990; Long *et al.* 1995; MacDonald *et al.* 1996; USEPA 1997b; CCME 1999; Field *et al.* 2002).

A review of the literature was conducted to identify chemical benchmarks that are relevant for evaluating the effects of sediment-associated COPCs on the microbial community. The results of this review indicated that SQGs have only infrequently been established explicitly for the protection of microorganisms that utilize benthic habitats. However, the Washington Department of Ecology has established such chemical benchmarks using the apparent effects threshold (AET) approach (Barrick *et al.* 1988). The Microtox™ AETs that were established by the Washington Department of Ecology were selected for assessing the risks to the microbial community posed by COPCs in whole sediments in the Calcasieu Estuary (Table C-2). Because the Microtox™ AETs represent the concentrations of sediment-associated contaminants above which adverse effects are always observed on the toxicity test organism (i.e., the bacterium, *Vibrio fisheri*), COPCs were considered to pose a significant risk to the microbial community at concentrations in excess of the Microtox™ AETs. That is, whole-sediment samples with concentrations of one or more COPCs above the Microtox AETs were predicted to be toxic to the microbial community. An evaluation of the predictive ability of the Microtox AETs is presented in Appendix E1.

3.6 Characterization of Risks to the Microbial Community

The purpose of risk characterization is to determine if significant effects are occurring or are likely to occur at the site under investigation. In addition, this step of the process is intended to provide the information needed to describe the nature, magnitude, and areal extent of effects on the selected assessment endpoints. Finally, the substances that are causing or substantially contributing to such effects (i.e., COCs) are identified from the COPCs. This information is generated by integrating the results of the exposure assessment with the results of the effects assessment, with each line of evidence initially considered separately. An evaluation of the uncertainty in the analysis provides a basis for determining the level of confidence that can be placed on these results and for integrating multiple lines of evidence into an overall assessment of risks to the microbial community. In the final step of the process, the various lines of evidence were considered together to establish a weight of evidence for assessing risks to the assessment endpoint under consideration.

To support the objectives of the risk characterization process, the results of Phase I and Phase II of the RI were compiled and used to assess risks to the microbial community associated with exposure to COPCs in sediment. Two lines of evidence were examined to determine if sediments in the Calcasieu Estuary pose significant risks to the microbial community, including whole-sediment chemistry and whole-sediment toxicity. Evaluation of the whole-sediment chemistry data collected during Phase I and Phase II of the RI using the selected toxicity thresholds for whole sediment (Table C-2) indicates that roughly 54% (337 of 624) of the sediment samples from the three AOCs have concentrations of COPCs that are sufficient to cause or substantially contribute to sediment toxicity to marine bacteria (Table C-27). By comparison, 15% of the whole-sediment samples (11 of 75 samples) collected from the three AOCs were found to be acutely toxic to marine bacteria (endpoint: EC_{50}-bioluminescence; Table C-28). When considered together, these two lines of evidence indicate that exposure to contaminated sediments is adversely affecting the metabolic rate of microorganisms in the Calcasieu Estuary and, hence, the activity of the microbial community.

3.6.1 Upper Calcasieu River Area of Concern

The Upper Calcasieu River includes the portion of the watershed from the saltwater barrier to the Highway 210 bridge, a distance of roughly 12 km (or 15 km, including the loop of the river located south of the saltwater barrier). This portion of the river system consists of several readily identifiable water bodies, including the Upper Calcasieu River mainstem from the saltwater barrier to Lake Charles, Lake Charles, Calcasieu Ship Channel from Lake Charles to the Highway 210 bridge, Clooney Island Loop, Contraband Bayou, Coon Island Loop, and Bayou Verdine (Figure C-2). The areas of interest within the UCR AOC include the Clooney Island Loop, Clooney Island Loop barge slip, Coon Island Loop northeast, and Coon Island Loop southwest (MacDonald *et al.* 2001). To facilitate assessment of risks to the microbial community, the UCR AOC was divided into four main reaches, including:

- Upper Calcasieu River - Mainstem and Calcasieu Ship Channel (i.e., from the saltwater barrier to upstream boundary of the BI AOC; Figure C-2);
- Clooney Island Loop (i.e., Figure C-2);
- Contraband Bayou (i.e., from the headwaters to the mouth; Figure C-2);

- Coon Island Loop (Figure C-2).

The risks to the microbial community posed by exposure to contaminated sediments were evaluated for each of these reaches and for the UCR AOC as a whole. Additionally, hot spots with respect to contaminated sediments were identified when possible.

3.6.1.1 Nature of Effects on the Microbial Community in the Upper Calcasieu River Area of Concern

Data on two measurement endpoints were used to determine if adverse effects on the microbial community were occurring in the UCR AOC in response to exposure to COPCs, including whole-sediment chemistry and whole-sediment toxicity. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to microbial communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring or are likely to be occurring within the study area, and to determine the nature of those effects.

When considered in conjunction with numerical SQGs (i.e., Microtox AETs; Table C-2), whole-sediment chemistry data provide a basis for evaluating the effects of contaminated sediments on microorganisms. The results of this evaluation indicate that roughly 25% (37 of 146) of the sediment samples from the UCR AOC have concentrations of COPCs that are sufficient to cause or substantially contribute to toxicity to microorganisms (i.e., compared to 12% for the reference areas; Table C-27). More specifically, these data demonstrate that sediment quality conditions sufficient to reduce the metabolic rate and, hence, activity of microorganisms exposed to contaminated sediments occur infrequently within the UCR AOC.

Based on the results of SPT with the bacterium, *Vibrio fisheri*, it is apparent that whole sediments from in the UCR AOC are adversely affecting the metabolic rate of microorganisms only infrequently. Of the 29 whole-sediment samples that were collected from the UCR AOC, a total of 3 (10%) were found to be toxic to marine bacteria (i.e., EC_{50} was lower than the 95% LCL for samples from reference areas; Table C-28). These data demonstrate that sediment quality conditions in the UCR AOC are sufficient to adversely affect the activity of the microbial community only infrequently.

When considered together, these two lines of evidence indicate that contaminated sediments generally pose low risks to the microbial community within the UCR AOC (i.e., the activity of the microbial community is likely to be adversely affected by exposure to contaminated sediments only infrequently). Therefore, it is concluded that significant effects on the microbial community are occurring only infrequently in the UCR AOC.

3.6.1.2 Magnitude of Effects on the Microbial Community in the Upper Calcasieu River Area of Concern

The magnitude of the effects on microorganisms exposed to contaminated sediments was evaluated using one line of evidence: whole-sediment toxicity. Based on the results of SPT with the bacterium, *Vibrio fisheri* (i.e., observed magnitude of toxicity), it is apparent that exposure to whole sediments from the UCR AOC is associated with a range of responses in microorganisms. Of the 29 whole-sediment samples that were collected from the UCR AOCs, a total of 26 (90%) were found to pose a low risk to marine bacteria (i.e., EC_{50}-bioluminescence values were similar to those observed for samples from reference areas; Table C-29). By comparison, the EC_{50}-bioluminescence was reduced by 10 to 20% in none of the samples (0%) and by >20% in three of the samples (10%) from the UCR AOC (Table C-29). Overall, the information on the observed magnitude of toxicity to microorganisms indicates that exposure to whole sediments from the UCR AOC generally poses a low risk to the microbial community (Table C-29). Nevertheless, the results of this evaluation demonstrate that this AOC has a number of hot spots with respect to sediment contamination and toxicity that may require further assessment and/or remedial action.

3.6.1.3 Preliminary Assessment of the Areal Extent of Effects on the Microbial Community in the Upper Calcasieu River Area of Concern

A preliminary assessment of the areal extent of adverse effects on microbial communities in the UCR AOC was conducted using the whole-sediment chemistry data that were collected in Phase I and Phase II of the RI. To support this evaluation of the spatial distribution of effects, each sediment sample was classified into one of three categories (i.e., low, indeterminate, or high) based on the risk that it posed to microorganisms. Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial Analyst software. The reaches that were considered in this analysis included the Upper Calcasieu River mainstem reach, Clooney Island Loop reach, Contraband Bayou reach, and Coon Island Loop reach.

Upper Calcasieu River - Mainstem Reach - Whole-sediment chemistry data are available for a total of 49 samples from the Upper Calcasieu River mainstem reach of the UCR AOC. Of these, five whole-sediment samples (10%) had concentrations of one or more COPCs that were sufficient to adversely affect the metabolic rate of bacteria (Table C-27). Hence, contaminated sediments were considered to generally pose a low risk to the microbial community. Based on the comparisons of whole sediment chemistry data to the Microtox AETs, contaminated sediments pose a high risk to microorganisms in Upper Calcasieu River downstream of the salt water barrier, the north central and south central portions of Lake Charles, and in the Calcasieu River downstream of Lake Charles (Figures C-6a and C-6b). The results of SPT with the bacterium, *Vibrio fisheri*, indicate that a whole-sediment sample from the eastern portion of Lake Charles, was toxic to the bacterium, *Vibrio fisheri* (Figure C-7).

Clooney Island Loop Reach - For the Clooney Island Loop reach of the UCR AOC, whole-sediment chemistry data are available for a total of 32 samples. Of these, 13 of the whole-sediment samples (41%) from this reach had COPC concentrations sufficient to adversely affect the metabolic rate of bacteria (Table C-27). Hence, contaminated sediments were considered to generally pose an indeterminate risk to the microbial community. The locations where contaminated sediments pose a high risk to microorganisms include Clooney Island barge slip, in small embayments in the northern and northeastern portion of the loop and, on the northwestern side of Clooney Island (Figures C-6a and C-6b). The results of SPT with the bacterium, *Vibrio fisheri*, indicate that a whole-sediment sample from the Clooney Island barge slip was toxic to microorganisms (Figure C-7).

Contraband Bayou Reach - Based on the results of chemical analysis of nine whole-sediment samples, it is apparent that sediment-associated contaminants pose a low risk to microorganisms in the Contraband Bayou reach of UCR. Comparison of the whole-sediment chemistry data to the toxicity thresholds for whole sediments (i.e., the Microtox AETs) indicates that one of the nine samples (11%) collected in this reach had COPC concentrations sufficient to adversely affect the microbial community (Table C-27). The sample with elevated levels of COPCs was collected downstream of the Prien Lake Road bridge (Figures C-6a and C-6b). None of the six samples tested from Contraband Bayou were found to be toxic to the bacterium, *Vibrio fisheri* (Figure C-7).

Coon Island Loop Reach - Fifty-six sediment samples have been collected from the Coon Island Loop reach of the AOC. Of these, 18 whole-sediment samples (32%) had COPC concentrations sufficient to adversely affect the microbial community (Table C-27). As such, the risks to the microbial community were generally classified as indeterminate. The hot spots with respect to sediment contamination (i.e., as indicated by exceedances of the Microtox AETs) include the mouth of Bayou Verdine, and throughout much of Coon Island Loop (Figures C-6a and C-6b). The results of whole-sediment toxicity tests indicate that a sediment sample from the southeast portion of Coon Island Loop was toxic to the bacterium, *Vibrio fisheri* (Figure C-7).

In summary, sediments within the UCR AOC are generally of sufficient quality to support the normal activity of the microbial community. Overall, the mean PEC-Qs calculated for the sediment samples collected from the UCR AOC have chemical characteristics that are similar to those that were measured in the reference areas (Table C-29). Additionally, the incidence and magnitude of toxicity to the bacterium, *Vibrio fisheri*, were similar to those that were observed for reference areas (Tables C-28 and C-29). Nevertheless, a number of hot spots with respect to sediment contamination were identified within the UCR AOC, with the highest risks to the microbial community occurring in the Upper Calcasieu River downstream of the salt water barrier, portions of Lake Charles, Calcasieu River downstream of Lake Charles, Clooney Island barge slip, the northern and eastern portions of Clooney Island Loop, Contraband Bayou downstream of the Prien Lake Road bridge, the mouth of Bayou Verdine, and throughout much of the Coon Island Loop.

3.6.1.4 Contaminants of Concern in the Upper Calcasieu River Area of Concern

Following the assessment of risks to the microbial community, it is important to identify the factors that are causing or substantially contributing to adverse effects on microorganisms. In this document, the substances that occur in UCR AOC sediments at concentrations that are sufficient to cause or substantially contribute adverse effects on the activity of the microbial community are termed contaminants of concern (COCs). The COCs in the UCR AOC, relative to the potential for adversely affecting microbial communities, were identified by comparing the concentrations of COPCs in whole sediments to the concentrations of those substances in reference sediments and to the selected benchmarks for those substances (Table C-2). Additionally, the distributions of the effects and no effects data for SPT with the bacterium, *Vibrio fisheri*, were examined to confirm that there was concordance between chemical concentrations and biological responses for each COC identified.

Based on the results of the exposure assessment, a total of 86 substances or groups of substances occurred in whole sediments from the UCR AOC at levels a factor of two or more higher than the 95% UCL for reference sediments (Table C-30). Subsequent screening against benchmarks for whole-sediment chemistry revealed that many of these substances represent preliminary COCs relative to the microbial community (Table C-31). Divergence of the cumulative effects and no effects distributions compiled using the matching sediment chemistry and laboratory toxicity data confirmed that the following substances are considered to be COCs in the UCR AOC (Table C-32; Figures C-8 to C-30): various individual PAHs; total LMW-PAHs; total PCBs; and, BEHP (Table C-33). Historic and/or ongoing sources of these substances are known to occur exist the estuary (see BPF for more information on the sources of these COCs; Appendix A). Insufficient information was available to determine if many other substances represented COCs, including chromium, various individual PAHs, various PCB congeners, certain phthalates, 1,2-dichloroethane, certain chlorinated benzenes, various PCDDs, acetone, and carbon disulfide (Table C-33).

3.6.2 Bayou d'Inde Area of Concern

Bayou d'Inde is one of the major tributaries to the Calcasieu River (Figure C-3). From its headwaters near Sulphur, Louisiana, Bayou d'Inde flows in a southeasterly direction some 16 km to its confluence with the Calcasieu Ship Channel (or roughly 11 km from the I-10 bridge to the mouth). Over that distance, Bayou d'Inde is joined by several tributaries, the largest of which is Maple Fork. The lower portions of the bayou are characterized by hydraulic connections (i.e., channels) with a great deal of off-channel wetland habitat, the largest of which is the Lockport Marsh. The areas of interest within the BI AOC include Lower Bayou d'Inde and Middle Bayou d'Inde (MacDonald *et al.* 2001). To facilitate assessment of risks to the microbial community, the BI AOC was divided into five reaches, including:

- Upper Bayou d'Inde (i.e., from the headwaters to the Highway 108 bridge; Figure C-3);
- Middle Bayou d'Inde (i.e., from the Highway 108 bridge to the confluence with PPG Canal; Figure C-3);

- Lower Bayou d'Inde (i.e., the mainstem from the confluence with PPG Canal to the confluence with the Calcasieu River; Figure C-3);
- PPG Canal (i.e., from the headwaters to the confluence with Bayou d'Inde; Figure C-3); and,
- Lockport Marsh (i.e., the wetland areas located near the mouth of Bayou d'Inde, but excluding the Lower Bayou d'Inde mainstem; Figure C-3).

The risks to the microbial community posed by exposure to contaminated sediments were evaluated for each of these reaches and for the BI AOC as a whole. Additionally, hot spots with respect to contaminated sediments were identified when possible.

3.6.2.1 Nature of Effects on the Microbial Community in the Bayou d'Inde Area of Concern

Data on two measurement endpoints were used to determine if adverse effects on the microbial community were occurring in the BI AOC in response to exposure to COPCs, including whole-sediment chemistry, and whole-sediment toxicity. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to microbial communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring or are likely to be occurring within the study area, and to determine the nature of those effects.

When considered in conjunction with numerical SQGs (i.e., Microtox AETs; Table C-2), whole-sediment chemistry data provide a basis for evaluating the effects of contaminated sediments on microorganisms. The results of this evaluation indicate that roughly 81% (254 of 315) of the sediment samples from the BI AOCs have concentrations of COPCs that are sufficient to cause or substantially contribute to toxicity to marine bacteria (i.e., compared to 12% for the reference areas; Table C-27). More specifically, these data demonstrate that sediment quality conditions in the BI AOC are frequently sufficient to reduce the metabolic rate and, hence, activity of microorganisms exposed to contaminated sediments.

Based on the results of SPT with the bacterium, *Vibrio fisheri*, it is apparent that whole sediments from the BI AOC are adversely affecting the metabolic rate of microorganisms only infrequently. Of the 31 whole-sediment samples that were collected from the BI AOC, a total of 5 (16%) were found to be toxic to marine bacteria (i.e., EC_{50} was lower than the 95% LCL for samples from reference areas; Table C-28). These data demonstrate that sediment quality conditions in the BI AOC are sufficient to adversely affect the activity of the microbial community at certain locations.

When considered together, these lines of evidence indicate that contaminated sediments pose variable risks to the microbial community. Nevertheless, information on both the predicted and observed incidence of toxicity indicates that significant effects on the microbial community are occurring in the BI AOC.

3.6.2.2 Magnitude of Effects on the Microbial Community in the Bayou d'Inde Area of Concern

The magnitude of the effects on microorganisms exposed to contaminated sediments was evaluated using one line of evidence: whole-sediment toxicity. Based on the results of SPT with the bacterium, *Vibrio fisheri* (i.e., observed magnitude of toxicity), it is apparent that exposure to whole sediments from the BI AOC is associated with a range of responses in microorganisms. Of the 31 whole-sediment samples that were collected from the BI AOCs, a total of 26 (84%) were found to pose a low risk to marine bacteria (i.e., the EC_{50}-bioluminescence values were similar to those observed for samples from reference areas; Table C-29). By comparison, the EC_{50}-bioluminescence was reduced by 10 to 20% in none of the samples (0%) and by >20% in five of the samples (16%) from the BI AOC. Overall, the information on the observed magnitude of toxicity to microorganisms indicates that exposure to whole sediments from the BI AOC generally poses a low risk to the microbial community (Table C-29). Nevertheless, the results of this evaluation demonstrate that this AOC has a number of hot spots with respect to sediment contamination and toxicity that may require remedial action.

3.6.2.3 Preliminary Assessment of the Areal Extent of Effects on the Microbial Community in the Bayou d'Inde Area of Concern

A preliminary assessment of the areal extent of adverse effects on microbial communities in the BI AOC was conducted using the whole-sediment chemistry data that were collected in Phase I and Phase II of the RI. To support this evaluation of the spatial distribution of chemical contamination, each sediment sample was classified into one of three categories (i.e., low, indeterminate, or high) based on the risk that it posed to microorganisms. Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial. The reaches that were considered in this analysis included Upper Bayou d'Inde, Middle Bayou d'Inde, Lower Bayou d'Inde mainstem, Lockport Marsh, and PPG Canal.

Upper Bayou d'Inde Reach - Whole-sediment chemistry data are available for a total of 53 samples from the Upper Bayou d'Inde reach of the BI AOC. Of these, 33 whole-sediment samples (62%) had concentrations of one or more COPCs that were sufficient to adversely affect the metabolic rate of bacteria (i.e., relative to reference areas; Table C-27). Hence, contaminated sediments were considered to generally pose a high risk to the microbial community. Based on comparisons of the whole-sediment chemistry data to the Microtox AETs, contaminated sediments pose a high risk to microorganisms from approximately 0.5 km upstream of the CitCon facility to the Highway 108 bridge (Figures C-31a and C-31b). The results of whole-sediment toxicity tests indicate that the whole-sediment samples from Upper Bayou d'Inde immediately south of the Highway I-10 bridge and those from immediately upstream of the Highway 108 bridge were toxic to the bacterium, *Vibrio fisheri* (Figure C-32).

Middle Bayou d'Inde Reach - For the Middle Bayou d'Inde reach of the BI AOC, whole-sediment chemistry data are available for a total of 93 samples. Of these, 90 of the whole-sediment samples (97%) from this reach had COPC concentrations sufficient to adversely affect the metabolic rate of bacteria (Table C-27). Hence, contaminated sediments were considered to pose a high risk to the microbial community. Based on the whole-sediment chemistry data, contaminated sediments pose a high risk to microorganisms throughout this reach of the BI AOC (Figures C-31a and C-31b). The results of whole-sediment toxicity tests indicate that one of the whole-sediment samples from the stations located in Maple Fork was toxic to the bacterium, *Vibrio fisheri* (Figure C-32).

Lower Bayou d'Inde Reach - Based on the results of chemical analysis of 38 whole-sediment samples, it is apparent that, sediment-associated contaminants generally pose a high risk to microorganisms in the mainstem portion of lower

Bayou d'Inde. Of these, 33 whole-sediment samples (87%) had COPC concentrations sufficient to adversely affect the microbial community (Table C-27). The samples with elevated levels of COPCs occurred throughout this reach of the BI AOC (Figures C-31a and C-31b). None of the samples (n=3) collected from this reach were found to be toxic to the bacterium, *Vibrio fisheri* (Figure C-32).

Lockport Marsh Reach - A total of 125 sediment samples were collected from the Lockport Marsh portion of Lower Bayou d'Inde. Comparison of the whole-sediment chemistry data to the toxicity thresholds for whole sediments (i.e., Microtox AETs) indicates that 92 of the 125 samples (74%) collected in this reach had concentrations of COPCs sufficient to adversely affect the microbial community (Table C-27). As such, risks to the microbial community were classified as high within this reach. The hot spots with respect to sediment contamination (i.e., as indicated by the exceedances of the Microtox AETs) occurred throughout much of Lockport Marsh, with the levels of risk lowest in the areas located closest to the Calcasieu Ship Channel (Figures C-31a and C-31b). The results of whole-sediment toxicity tests indicated that none of the sediment samples from Lockport Marsh were toxic to the bacterium, *Vibrio fisheri* (Figure C-32).

PPG Canal Reach - Fewer sediment samples were collected from PPG Canal (n=6) than were collected from the other reaches within the BI AOC. All six of these samples were predicted to be toxic to microorganisms, based on comparisons of the whole-sediment chemistry data to the Microtox AETs (Table C-27). Hence, the risks posed by COPCs to the microbial community were considered to be high throughout this reach (Figures C-31a and C-31b). Based on the results of SPT with the bacterium, *Vibrio fisheri*, one of these six sediment samples (17%) was found to be toxic to microorganisms (i.e., the sample collected from the site located closest to the mouth of the canal; Figure C-32).

In summary, sediments within the BI AOC pose risks to the microbial community ranging from low to high, depending on the line of evidence that was considered. The mean PEC-Qs calculated for the sediment samples collected from this AOC were higher than those that were measured in the reference areas, indicating that the levels of COPCs are generally higher in the BI AOC than they are in reference areas (Table C-29). Accordingly, the predicted incidence of toxicity to microorganisms is high throughout this reach. Additionally, the observed incidence of toxicity to the bacterium, *Vibrio fisheri*, was elevated in three of the five reaches relative to that for reference areas. Importantly, a number of hot spots with respect to sediment contamination were identified within the BI AOC, with the highest risks to microorganisms occurring in portions of Upper Bayou d'Inde, and throughout Middle Bayou d'Inde, PPG Canal, the Lower Bayou d'Inde mainstem, and Lockport Marsh. As such, the activity of the microbial community is likely to be impaired throughout much of BI AOC, particularly in those areas located downstream of the CitCon facility (i.e., the lower 8 km of Bayou d'Inde and associated tributaries).

3.6.2.4 Contaminants of Concern in the Bayou d'Inde Area of Concern

Following the assessment of risks to the microbial community, it is important to identify the factors that are causing or substantially contributing to adverse effects on microorganisms. In this document, the substances that occur in BI AOC sediments at concentrations that are sufficient to cause or substantially contribute to adverse effects on the activity of the microbial community are termed contaminants of concern (COCs). The COCs in the BI AOC, relative to the potential for adversely affecting microbial communities, were identified by comparing the concentrations of COPCs in whole sediments to the concentrations of those substances in reference sediments and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for the SPT with the bacterium, *Vibrio fisheri*, were examined to confirm that there was concordance between chemical concentrations and biological responses for each COC identified.

Based on the results of the exposure assessment, a total of 94 substances or groups of substances occurred in whole sediments from the BI AOC at levels above the 95% UCL for reference sediments (Table C-34). Subsequent screening against benchmarks for whole-sediment chemistry revealed that many of these substances represent preliminary COCs relative to the microbial community (Table C-35). Divergence of the cumulative effects and no effects distributions compiled using the matching sediment chemistry and laboratory toxicity data confirmed the following substances represent COCs in the BI AOC (Table C-32; Figures C-8 to C-30): 1,1-biphenyl, various individual PAHs; total PCBs; and, BEHP (Table C-36). Historic and/or ongoing sources of these substances are known to exist in the estuary (see BPF for more information on the sources of these COCs; Appendix A). Insufficient information was available to determine if many other substances represented COCs, including chromium, various individual PAHs, various PCB congeners, certain phthalates, various chlorinated benzenes, 1,1,1-trichloroethane, 1,2-dichloroethane, various PCDDs, acetone, and carbon disulfide (Table C-36).

3.6.3 Middle Calcasieu River Area of Concern

The Middle Calcasieu River comprises the portion of the watershed from the Highway 210 bridge to the outlet of Moss Lake (a distance of roughly 12 km), excluding Bayou d'Inde (Figures C-4a and C-4b). The primary physiographic features in this portion of the study area include the Calcasieu Ship Channel, Prien Lake, the original Calcasieu River channel, and Moss Lake. For this assessment, the Indian Wells Lagoon and Bayou Olsen were also included in the Middle Calcasieu River study area. The areas of interest within the MCR AOC include south Prien Lake and the Indian Wells Lagoon outflow (MacDonald *et al.* 2001). To facilitate assessment of risks to the community, the MCR AOC was divided into five reaches, including:

- Middle Calcasieu River - Mainstem (i.e., Calcasieu Ship Channel and the old river channel, to the outlet of Moss Lake, excluding the portion of the channel south of Prien Lake; Figure C-4a);
- Prien Lake and the upper old river channel (Figure C-4a);
- Indian Wells Lagoon (Figure C-4a);
- Bayou Olsen (i.e., from the headwaters to the mouth; Figure C-4b); and,
- Moss Lake (i.e., excluding the Calcasieu Ship Channel; Figure C-4b).

The risks to the microbial community posed by exposure to contaminated sediments were evaluated for each of these reaches and for the MCR AOC as a whole. Additionally, hot spots with respect to contaminated sediments were identified when possible.

3.6.3.1 Nature of Effects on the Microbial Community in the Middle Calcasieu Area of Concern

Data on two measurement endpoints were used to determine if adverse effects on the microbial community were occurring in the MCR AOC in response to exposure to

COPCs, including whole-sediment chemistry and whole-sediment toxicity. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to microbial communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring or are likely to be occurring within the study area, and to determine the nature of those effects.

When considered in conjunction with numerical SQGs (i.e., Microtox AETs; Table C-2), whole-sediment chemistry data provide a basis for evaluating the effects of contaminated sediments on microorganisms. The results of this evaluation indicate that roughly 28% (46 of 163) of the sediment samples from the MCR AOCs have concentrations of COPCs that are sufficient to cause or substantially contribute to toxicity to marine bacteria (i.e., compared to 12% for the reference areas; Table C-27). These data demonstrate that sediment quality conditions in the MCR AOC are sufficient to reduce the metabolic rate and, hence, activity of microorganisms exposed to contaminated sediments.

Based on the results of SPT with the bacterium, *Vibrio fisheri*, it is apparent that whole sediments from the MCR AOC are adversely affecting the metabolic rate of microorganisms. Of the 15 whole-sediment samples that were collected from the MCR AOCs, a total of three (20%) were found to be toxic to marine bacteria (i.e., EC_{50} was lower than the 95% LCL for samples from reference areas; Table C-28). These data demonstrate that sediment quality conditions in portions of the MCR AOC are sufficient to adversely affect the activity of the microbial community.

When considered together, these lines of evidence indicate that contaminated sediments pose variable risks to the microbial community (i.e., the activity of the microbial community is likely to be adversely affected at various locations by exposure to contaminated sediments). Therefore, it is concluded that significant effects on the microbial community are occurring throughout portions of the MCR AOC.

3.6.3.2 Magnitude of Effects on the Microbial Community in the Middle Calcasieu River Area of Concern

The magnitude of the effects on microorganisms exposed to contaminated sediments was evaluated using one line of evidence: whole-sediment toxicity. Based on the results of SPT with the bacterium, *Vibrio fisheri* (i.e., observed magnitude of toxicity), it is apparent that exposure to whole sediments from the MCR AOC is associated with a range of responses in microorganisms. Of the 15 whole-sediment samples that were collected from the MCR AOC, a total of 12 (80%) were found to pose a low risk to the microbial community (i.e., the EC_{50}-bioluminescence values were similar to those observed for samples from reference areas; Table C-29). By comparison, the EC_{50}-bioluminescence was reduced by 10 to 20% in none of the samples (0%) and by >20% in three of the samples (20%) from the MCR AOC. Overall, the information on the observed magnitude of toxicity to microorganisms indicates that exposure to whole sediments from the MCR AOC generally poses a low risk to the microbial community (Table C-29). Nevertheless, the results of this evaluation demonstrate that this AOC has a number of hot spots with respect to sediment contamination and toxicity that may require remedial action.

3.6.3.3 Preliminary Assessment of the Areal Extent of Effects on the Microbial Community in the Middle Calcasieu River Area of Concern

A preliminary assessment of the areal extent of adverse effects on microbial communities in the MCR AOC was conducted using the whole-sediment chemistry data that were collected in Phase I and Phase II of the RI. To support this evaluation of the spatial distribution of chemical contamination, each sediment sample was classified into one of three categories (i.e., low, indeterminate, or high), based on the risk that it posed to microorganisms. Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial Analyst software. The reaches that were considered in this analysis included the Middle Calcasieu River mainstem, Prien Lake and the upper old river channel, Indian Wells Lagoon, Bayou Olsen, and Moss Lake.

Middle Calcasieu River - Mainstem Reach - Whole-sediment chemistry data are available for a total of 76 samples from the Middle Calcasieu River mainstem reach of the MCR AOC. Of these, 20 whole-sediment samples (26%) had concentrations of one or more COPCs that were sufficient to adversely affect the metabolic rate of bacteria (i.e., relative to reference areas; Table C-27). Hence, whole sediments were considered to generally pose a low risk to the microbial community. Based on comparisons of the whole-sediment chemistry data to the Microtox AETs, sediments along the western shoreline of the Middle Calcasieu River from the mouth of Bayou d'Inde to Moss Lake frequently pose a high risk to the microbial community (Figures C-33a, C-33b, and C-33c). No samples were collected to support toxicity testing of whole sediments from this reach.

Prien Lake and Upper Old River Channel Reach - For the Prien Lake and upper old river channel reach of the MCR AOC, whole-sediment chemistry data are available for a total of 49 samples. Of these, eight whole-sediment samples (16%) had mean COPC concentrations sufficient to adversely affect the microbial community (Table C-27). The locations where contaminated sediments pose a high risk to microorganisms include: the western and southeastern portions of Prien Lake, and the western portion of the old river channel (Figures C-33a and C-33b). The results of whole-sediment toxicity tests indicate that none of the whole-sediment samples (n=4) from this reach were toxic to the bacterium, *Vibrio fisheri* (Figure C-34a).

Indian Wells Lagoon Reach - Based on the results of chemical analysis of 10 whole-sediment samples, it is apparent that sediment-associated contaminants generally pose a high risk to microorganisms in the Indian Wells Lagoon reach of MCR. Of these, nine whole-sediment samples (90%) had COPC concentrations sufficient to adversely affect the microbial community (Table C-27). Based on comparisons of the whole-sediment chemistry data to the Microtox AETs, it is apparent that sediments throughout this reach are sufficiently contaminated to pose high risks to the microbial community (Figures C-33a and C-33b). One of the samples (n=3) collected from Indian Wells Lagoon was found to be toxic to the bacterium, *Vibrio fisheri* (Figure C-34a).

Bayou Olsen Reach - Based on the available whole-sediment chemistry data, Bayou Olsen was among the least contaminated reaches of the MCR AOC. As such, risks to the microbial community were classified as low within this reach (Table C-27). Evaluation of these whole-sediment chemistry data on a sample by sample basis indicates that the concentrations of COPCs are likely to pose low risks to microorganisms throughout this reach (i.e., 0 of 11 samples were predicted to be toxic; Figure C-33c). In addition, the results of SPT with the bacterium, *Vibrio fisheri*, indicate that none of the sediment samples (n=5) from this reach are toxic to the bacterium, *Vibrio fisheri* (Figure C-34b).

Moss Lake Reach - For Moss Lake, whole-sediment chemistry data are available for 17 sediment samples. Of these, nine sediment samples (53%) were predicted to be toxic to microorganisms, based on comparisons of the whole-sediment chemistry data to the Microtox AETs (Table C-27). The concentrations of sediment-associated COPCs are sufficient to pose high risks to the microbial community throughout the central and western portions of Moss Lake (Figure C-33c). Of the three whole-sediment samples that were tested for this reach, two were found to be toxic to the bacterium, *Vibrio fisheri* (Figure C-34b).

In summary, sediments within the MCR AOC are generally of sufficient quality to support the normal activity of the microbial community. Overall, the mean PEC-Qs calculated (0.160) indicate that the sediment samples collected from this reach have chemical characteristics that are generally similar to those that were measured in the reference areas (0.106; Table C-29). However, roughly 28% of the sediment samples from the MCR AOC have contaminant concentrations sufficient to adversely affect the microbial community (Table C-27). The hot spots with respect to sediment contamination are largely associated with the Middle Calcasieu River mainstem, western and southeastern portions of Prien Lake, Moss Lake, and the Indian Wells Lagoon reaches of the MCR AOC.

3.6.3.4 Contaminants of Concern in the Middle Calcasieu River Area of Concern

Following the assessment of risks to the microbial community, it is important to identify the factors that are causing or substantially contributing to adverse effects on microorganisms. In this document, the substances that occur in MCR AOC sediments at concentrations that are sufficient to cause or substantially contribute adverse effects on the activity of the microbial community are termed contaminants of concern (COCs). The COCs in the MCR AOC, relative to the potential for adversely affecting microbial communities, were identified by comparing the concentrations of COPCs in whole sediments to the concentrations of those substances in reference sediments and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for the SPT with the bacterium, *Vibrio fisheri*, were examined to confirm that there was concordance between chemical concentrations and biological responses for each COC identified.

Based on the results of the exposure assessment, a total of 86 substances or groups of substances occurred in whole sediments from the MCR AOC at levels above the 95% UCL for reference sediments (Table C-37). Subsequent screening against benchmarks for whole-sediment chemistry revealed that the majority of these substances represent preliminary COCs relative to the microbial community (Table C-38). Divergence of the cumulative effects and no effects distributions compiled using the matching sediment chemistry and laboratory toxicity data confirmed that the substances that represent COCs in the MCR AOC (Table C-32; Figures C-8 to C-30) include: numerous individual PAHs; total LMW-PAHs; total HMW-PAHs; total PAHs; total PCBs; and, BEHP (Table C-39). Historic and/or ongoing sources of all three of these substances are known to exist in the estuary (see BPF for more information on the sources of these COCs; Appendix A). Insufficient information was available to determine if many other substances represented COCs, including chromium, 1,1-biphenyl, various individual PAHs, various PCB congeners, certain phthalates, 1,2-dichloroethane, various chlorinated benzenes, various PCDDs, acetone, and carbon disulfide (Table C-39).

4.0 Uncertainty Analysis

There are a number of sources of uncertainty in assessments of risk to the microbial community, including uncertainties in the conceptual model, in the exposure assessment, and in the effects assessment. As each of these sources of uncertainty can influence the estimations of risk, it is important to describe and, when possible, quantify the magnitude and direction of such uncertainties. The purpose of this section is to evaluate uncertainty in a manner that facilitates attribution of the level of confidence that can be placed in the assessments conducted using the various lines of evidence. Accordingly, the uncertainties associated with the assessment of risks to microbial communities are described in the following sections.

4.1 Uncertainties Associated with the Conceptual Model

The conceptual model is intended to define the linkages between stressors, potential exposure, and predicted effects on ecological receptors. As such, the conceptual model provides the scientific basis for selecting assessment and measurement endpoints to support the risk assessment process. Potential uncertainties arise from the lack of knowledge regarding ecosystem functions, failure to adequately address spatial and temporal variability in the evaluations of sources, fate, and effects, omission of stressors, and overlooking secondary effects (USEPA 1998). The potential sources of uncertainty that are associated with the conceptual model that links contaminant sources to effects on the microbial community include those associated with the identification of COPCs, environmental fate and transport of COPCs, exposure pathways, receptors at risk, and ecological effects. Of these, the identification of exposure pathways probably represents the primary source of uncertainty in the conceptual model.

In this assessment, it was assumed that exposure to whole sediments represents the most important pathway for exposing microbial communities to COPCs (i.e., as the microorganisms associated with benthic habitats likely play the most important role in key ecological functions and contaminant concentrations are likely to be highest in these media types). However, microorganisms may also be exposed to COPCs in surface water and pore water. As such, risks to the microbial community could be under-estimated if these pathways resulted in significant exposure of microorganisms to COPCs. Because these pathways are considered for other receptor groups (i.e., pore water for plants, benthic invertebrates, and fish; Appendices D, E2, and F1 respectively; surface water for plants and fish; Appendix D and F, respectively) and the benchmarks used to assess risks to various receptor groups are similar, the risks to the microbial community associated with exposure to surface water or pore water can be inferred from the results for the other assessments.

4.2 Uncertainties Associated with the Exposure Assessment

The exposure assessment is intended to describe the actual or potential co-occurrence of stressors with receptors. As such, the exposure assessment identifies the exposure pathways and the intensity and extent of contact with stressors for each receptor or group of receptors at risk. There are a number of potential sources of uncertainty in the exposure assessment, including measurement errors, extrapolation errors, and data gaps.

In this assessment, two types of measurements were used to evaluate exposure of the microbial community to COPCs, including chemical analyses of environmental media (i.e., whole sediment) and toxicity tests conducted using indicator species. Relative to the whole-sediment chemistry data, analytical errors and descriptive errors represent potential sources of uncertainty. Three approaches were used to address concerns relative to these sources of uncertainty. First, analytical errors were evaluated using information on the accuracy, precision, and detection limits (DL) that were generated to support the Phase I and Phase II sampling programs. The results of this analysis indicated that most of the data used in this assessment met the project data quality objectives (see Appendix B1 for more details). Second, all data entry, data translation, and data manipulations were audited to assure their accuracy. Data auditing involved 10% number-for-number checks against the primary data source initially, increasing to 100% number-for-number checks if significant errors were detected in the initial auditing step. Finally, statistical analyses of resultant data were conducted to evaluate data distributions, identify the appropriate summary statistics to generate, and evaluate the variability in the observations. As such, measurement errors in the whole-sediment chemistry data are considered to be of minor importance and are unlikely to substantially influence the results of the risk assessment.

The treatment of whole-sediment chemistry data has the potential to influence the results of the BERA. In particular, the treatment of less than detection limit data can effect the results of the exposure assessment and the risk characterization. A number of investigators have evaluated the implications of applying various procedures for estimating the concentrations of COPCs from less than detection limit data (Gaskin *et al.* 1990; Porter and Ward 1991; El-Shaawari and Esterby 1992; Clarke and Brandon 1994). While there is no consensus on which data censoring method should be used in various applications, the simplest methods tend to be used most frequently, including deletion of non-detect values or substitution of a constant, such as zero, the detection limit, or one-half the detection limit (USACE 1995).

To address the need for guidelines for statistical treatment of less than detection limit data, the United States Army Corps of Engineers (USACE 1995) conducted a simulation study to assess the performance of 10 methods for censoring data. The results of that investigation indicated that no single data censoring methods works best in all situations. Accordingly, the USACE (1995) recommended a variety of methods depending on the proportion of the data that requires censoring, the distribution and variance of the data, and the type of data transformation. For data sets for which a low to indeterminate proportion of the data require censoring, substitution of the detection limit is generally the preferred methods (i.e., to optimize statistical power and control type I error rates). However, as the proportion of the data that requires censoring and the coefficient of variation of the data increase, statistical power is better maintained by substituting one-half of the detection for the less than detection limit data, particularly for lognormally distributed and transformed data. Substitution of zero or other constants was also recommended for a variety of circumstances. Overall, it was concluded that simple substitution methods work best to maintain power and control error rates in statistical comparisons of chemical concentration data (USACE 1995).

In this analysis, decisions regarding the treatment of less than detection limit data were made by considering the recommendations that have emerged from previous investigations in the context of their potential effects on the results of the BERA. Including all of the whole-sediment chemistry data that were collected in the

Calcasieu Estuary RI, roughly 80% of the data required censoring prior to data analysis. To minimize the potential effects of the less than detection limit data on the results of the BERA, none of the less than detection limit data, for which the detection limits were greater than the corresponding toxicity thresholds for whole-sediment chemistry (i.e., benchmarks) was used in the exposure analysis. Consistent with the guidance developed by USACE (1995), one-half of the detection limit was substituted for all of the other less than detection limit data. This procedure facilitated estimation of distributions of the concentrations of COPCs and eliminated the potential for identifying significant risks based on less than detection limit data.

Selection of an alternate procedure for treating the less than detection limit data has the potential for influencing the results of the BERA. For example, substitution of zero for less than detection limit data would have altered the distributions of the COPC concentration data for the three AOCs and the reference area (i.e., the estimated 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentile concentrations would likely have been lower than the estimates developed for the exposure assessment). Likewise, substitution of the detection limit for the less than detection limit data would have altered the distributions of the COPC concentration data for the three AOCs and the reference area (i.e., the estimated 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentile concentrations would likely have been higher than the estimates developed for the exposure assessment). Although the influence of these alternate methods on the estimate of the 75th or 95th percentile concentration would likely have been minor, their selection could have influenced the identification of COCs for one or more AOCs. However, neither the nature, magnitude, nor areal distribution of risks to microbial communities was affected by the selection of data treatment methods. As such, the potential impact of the methods that were selected for treating less than detection limit data on the results of the BERA are considered to be minor.

Data gaps also represent a potential source of uncertainty in the assessments of exposure for ecological receptors. However, whole-sediment chemistry data are available for more than 600 stations located throughout the Calcasieu Estuary. Therefore, limitations on the availability of whole-sediment chemistry data are not considered to materially affect the results of the BERA. Nevertheless, limitations on the availability of data on certain COPCs (e.g., chromium, various individual PAHs, certain chlorinated benzenes, and several PCDDs/PCDFs) restrict the identification of COCs in the various AOCs and in the estuary as a whole.

4.3 Uncertainties in the Effects Assessment

The effects assessment is intended to describe the effects that are caused by stressors, link them to the assessment endpoints, and evaluate how effects change with fluctuations in the levels (i.e., concentrations) of the various stressors. There are several potential sources of uncertainty in the assessment of effects on aquatic receptors, including measurement errors, extrapolation errors, and data gaps.

In this investigation, the effects on microbial communities associated with exposure to sediment-associated COPCs were evaluated using two types of information, including toxicity benchmarks for whole-sediment chemistry and whole-sediment toxicity tests. Although the toxicity benchmarks are not subject to measurement errors, the toxicity tests are. For this reason, potential measurement errors associated with toxicity tests were evaluated in the uncertainty analysis. More specifically, the data on negative controls and positive controls were examined to identify potential measurement errors. In addition, the results obtained from samples collected in the reference areas were considered in this analysis. More specifically, a reference envelope approach was used to classify whole-sediment samples as toxic or not toxic.

Because this approach facilitated the determination of the normal range of responses for samples from reference areas and only samples for which the response was beyond the 95% lower confidence limit (LCL; i.e., 25th percentile) were designated as toxic, the probability of incorrectly classifying a not toxic sample as toxic is roughly 0.025. However, the probability of incorrectly classifying a toxic sample as not toxic is probably higher. Therefore, application of the reference envelope approach may tend to underestimate risks to the microbial community.

There are several sources of extrapolation errors in the effects assessment for the Calcasieu Estuary BERA. First, the Microtox AETs were selected as the toxicity benchmarks for whole sediments for this assessment (Barrick *et al*. 1988). These SQGs were developed using matching sediment chemistry and elutriate toxicity data for Puget Sound, Washington. Based on the results of the predictive ability evaluation that was conducted as part of this investigation (Appendix E1), it is apparent that application of the Microtox AETs could result in over-estimating toxicity to marine bacteria, *Vibrio fisheri*, in the Calcasieu Estuary. However, the estuary-specific concentration-response relationships developed using a chemical mixture model (Appendix E1) suggest that marine bacteria are as or more sensitive to the effects of metals, PAHs, and PCBs than benthic invertebrates (i.e., based on comparison of the P_{50} values). The results of the overall assessments of risks to these two receptor groups reflect the relative sensitivities of microorganisms and benthic invertebrates. Therefore, it is unlikely that application of the Microtox AET resulted in gross overestimation of risks to the microbial community. Second, risks to the microbial community were evaluated based on observed and predicted effects on the bacterium, *Vibrio fisheri*. More specifically, light emission (i.e., bioluminescence) of *Vibrio fisheri*, was used as a surrogate for the metabolic rate of bacteria exposed to whole sediments in the Calcasieu Estuary (i.e., to provide a basis for determining if the essential functions that are performed by the microbial community, such as carbon processing, nitrogen cycling, and phosphorus cycling, are likely to be impaired). However, reductions in light emission can occur as a result of either functional responses (i.e., changes in metabolic rate) or reductions in survival. As such, the magnitude of the effects on the microbial community could be underestimated using the selected interpretation of the toxicity test results. That the selected benchmarks are based on the results of similar toxicity tests suggests that application of the Microtox AETs could result in an underestimate of the magnitude of effects. Although studies are currently underway to evaluate the relationship between the results of SPT with the bacterium, *Vibrio fisheri*, and the response of *in situ* microbial communities (Johnson *et al.* 2002a; 2002b; Nipper *et al*. 2002), insufficient information is currently available to determine if the selected approach is likely to under or over-estimate effects on the microbial community in the field.

Uncertainty in the effects assessment for aquatic receptors can also be increased by data gaps. To the extent possible, the use of multiple lines of evidence provides a basis for minimizing the influence of data gaps on the effects assessment. Nevertheless, limitations on certain types of data, such as concentration-response data for microbial species, makes it difficult to fully evaluate the effects of COPC exposures on microbial communities. For this reason, the present assessment could over-estimate or under-estimate risks to the microbial community.

5.0 Integrated Assessment of Risks to the Microbial Community in the Calcasieu Estuary Using a Weight of Evidence Approach

Information on two lines of evidence was compiled to support the assessment of risks to the microbial community associated with exposure to contaminated sediments in the Calcasieu Estuary. The previous sections of this appendix present the information for each of these individual lines of evidence and interpret that information to evaluate effects on the activity of microorganisms in the Calcasieu Estuary. As such, the previous evaluations were used to provide the information needed to determine if adverse effects on microorganisms are occurring or are likely to be occurring within the Calcasieu Estuary, to evaluate the nature, severity, and areal extent of such effects, and to identify the substances that are causing or substantially contributing to effects on the microbial community.

Each of the lines of evidence that was used in the assessment of risks to microbial communities has certain strengths and limitations that influence its application in the risk assessment process. For this reason, an uncertainty analysis was conducted to evaluate the level of confidence that can be placed in the results of analyses conducted using the individual lines of evidence (Section 4.0). Importantly, the uncertainty associated with the overall assessment of risks can be reduced by integrating information from multiple lines of evidence using a weight of evidence approach (Ingersoll and MacDonald 2002).

In this investigation, a simple arithmetic procedure was used to integrate information from multiple lines of evidence. In the first step of this process, the level of confidence (i.e., weight; as quantified by calculating a TES; Table C-40) that could be placed in each measurement endpoint was scored from one (low) to three (high) determined based on the following considerations (i.e., adapted from Suter *et al.* 2000):

Conceptual Model:

- *Relevance of Exposure Pathway:* Evidence was given more weight if the exposure pathway examined was the most relevant for assessing the status of the assessment endpoint. For example, exposure of aquatic plants to surface water would be considered to be more relevant than exposure to whole sediments; and,
- *Relevance of the Measurement Endpoint*: Evidence was given more weight if the measurement endpoint provided a direct estimate of the status of the assessment endpoint or if validation studies have demonstrated that measurement endpoint is predictive of the assessment endpoint. For example, measurement of the survival and growth of amphipods is considered to provide direct evidence for evaluating the survival and growth of benthic invertebrates. By comparison, data on the fertilization and development of sea urchin gametes and embryos (which are pelagic life history stages) is considered to provide less direct evidence for evaluating the reproduction of benthic invertebrates.

Exposure Assessment:

- *Level of Standardization*: Evidence was given more weight if standard methods were available and appropriately implemented to generate the data on exposure of receptors at risk to COPCs. For example, the level of standardization for surface-water chemistry and whole-sediment chemistry would be high because standard methods were used to collect, handle, transport, store, and analyse samples. By comparison, the level of standardization for pore-water chemistry would be lower because standard methods for collecting and processing samples have not been described;

- *Quality of Data:* Evidence was given more weight if the data were demonstrated to be of high quality. In this context, data quality was evaluated by considering the project data quality objectives and consider such criteria as accuracy, precision, and analytical detection limits;
- *Quantity of Data:* Evidence was given more weight if the sample size was considered to be adequate to characterize conditions within the study area;
- *Level of Temporal Coverage:* Evidence was given more weight if the data encompasses the relevant range of temporal variance of conditions. For example, a single sampling event was considered to evaluate characterize whole sediment chemistry because such conditions are unlikely to change substantially on a seasonal basis. In contrast, surface-water chemistry is likely to change on daily and seasonal bases, emphasizing the need for more comprehensive data sets to characterize temporal variability; and,
- *Level of Spatial Coverage:* Evidence was given more weight if the data adequately represented the geographic area that was being assessed. In this context, a measurement endpoint was scored high if samples were available from most or all of the areas of concern and associated reaches.

Effects Assessment:

- *Level of Standardization*: Evidence was given more weight if standard methods were available and appropriately implemented to generate the data on the effects associated with exposure of receptors at risk to COPCs. For example, concentration-response relationships were considered to be stronger if the underlying toxicity tests and chemical analyses were conducted using standard methods;

- *Meets Acceptability Criteria*: Evidence was given more weight if the established acceptability criteria for the measurement endpoint were met. For example, toxicity test results were weighted high if the negative and positive control results were with acceptable ranges;
- *Demonstrated Concentration - Response Relationship:* Evidence was given more weight if it demonstrated a relationship between the magnitude of exposure and the effects;
- *Relevance of the Exposure Medium*: Evidence was given more weight if the medium (i.e., surface water, sediment) considered in the assessment was consistent with the mode of exposure for the site medium. For example, the results of pore-water toxicity tests would be less relevant for assessing the status of the aquatic plant community than would the results of surface-water toxicity tests;
- *Level of Field Validation:* Evidence was given more weight if the results of validation studies have demonstrated that the measurement endpoint provides a reliable basis estimating the status of the assessment endpoint or the status of other measurement endpoints that are predictive of the assessment endpoint.

Consideration of the result that was obtained for each measurement endpoint (e.g., observed incidence of toxicity to marine amphipods, which was score 0, 1, or 2), in conjunction with the weight (i.e., TES) that was assigned to that measurement endpoint (which was scored from 1 to 3), provided a basis for developing a risk score for each measurement endpoint and line of evidence (Section 2.5). Subsequently, a final risk score was calculated by averaging the risk score for each line of evidence.

The final risk score is intended to provide an integrated measure of the risks that contaminated sediments pose to the activity of microbial communities in the Calcasieu

Estuary. More specifically, the final risk score integrates the results of whole-sediment toxicity tests and whole-sediment chemical analyses into a single parameter. The results of the integrated assessment of risks to microorganisms in the Calcasieu Estuary are presented in the following sections of this appendix.

5.1 Integrated Assessment of Risks to the Microbial Community in the Upper Calcasieu River Area of Concern

A total of 146 sediment samples were collected within the UCR AOC to support an assessment of the risks posed to the microbial community associated with exposure to sediment-associated COPCs. The results of this assessment indicated that exposure to whole sediments in the UCR AOC generally posed a low risk to microorganisms (i.e., average of the final risk scores of 0.727; n=146). Seventy-eight percent of the sediment samples (i.e., 114 of 146) from this AOC had low final risk scores (i.e., < 2; Table C-41). However, indeterminate risks to microorganisms were indicated for 22% of the sediment samples (32 of 146) of the sediment samples from the UCR AOC (Table C-41). Consistent with the results of the previous analyses, the locations where contaminated sediments posed the highest risks (i.e., relative to the activity of the microbial community) included the Calcasieu River downstream of the salt water barrier, portions of Lake Charles, the Calcasieu River downstream of Lake Charles, the Clooney Island barge slip, the northern and northeastern portions of Clooney Island Loop, and, the northern, central, and southern portions of the Coon Island Loop (Figures C-35a and C-35b).

The biological conditions that occur within the three risk categories indicate that sediments posing indeterminate and high risks are more toxic to the bacterium, *Vibrio fisheri*, than are sediments posing low risks (Table C-42). The mean EC_{50}-

bioluminescence was 11.1±8.4 for the whole-sediment samples that were classified into the low risk category (n=84). By comparison, the mean EC_{50}-bioluminescence was 0.50±0.32 (n=5) for the sediment samples that were classified into the indeterminate category. No samples were classified into the high risk category, so it was not possible to determine the average EC_{50} for this risk category. Therefore, the sediment samples included in the indeterminate and high risk categories have concentrations of COCs that are sufficient to adversely affect the metabolic rate of microorganisms and, as a result, the activity of the microbial community. Because many of the COCs in the UCR AOC are considered to be relatively persistent in surficial and deeper sediments and there is little evidence that COC concentrations are decreasing over time (see Table E1-7 of Appendix E1), it is likely that such effects will continue to impact the microbial community unless corrective action is taken to reduce risks in high (and possibly certain indeterminate) risk locations.

5.2 Integrated Assessment of Risks to the Microbial Community in the Bayou d'Inde Area of Concern

A total of 315 sediment samples were collected within the BI AOC to support an assessment of the risks posed to the microbial community associated with exposure to sediment-associated COPCs. The results of this assessment indicated that exposure to whole sediments in the BI AOC generally posed an indeterminate risk to microorganisms (i.e., average of the final risk scores of 2.32; n=315). Roughly 26% of the sediment samples (i.e., 83 of 315) from this AOC had low final risk scores (i.e., < 2; Table C-41). By comparison indeterminate risks to microorganisms were indicated for 74% (232 of 315) of the sediment samples from the BI AOC (Table C-41). Consistent with the results of the preliminary analysis of the areal extent of effects, the locations where contaminated sediments posed the highest risks (i.e., relative to the activity of the microbial community) included Upper Bayou d'Inde from roughly 0.5 km upstream of the CitCon property to the Highway 108 bridge, the mainstem and off-channel wetland areas throughout Middle Bayou d'Inde, near the mouth of PPG Canal, Lockport Marsh, the wetland areas located east of Lower Bayou d'Inde mainstem, and Lower Bayou d'Inde mainstem (Figures C-36a and C-36b).

The biological conditions that occur within the three risk categories indicate that sediments posing indeterminate and high risks are more toxic to the bacterium, *Vibrio fisheri*, than are sediments posing low risks (Table C-42). The mean EC_{50}-bioluminescence was 11.1±8.4 for the whole-sediment samples that were classified into the low risk category (n=84). By comparison, the mean EC_{50}-bioluminescence was 0.50±0.32 (n=5) for the sediment samples that were classified into the indeterminate category. No samples were classified into the high risk category, so it was not possible to determine the average EC_{50} for this risk category. Therefore, the sediment samples included in the indeterminate and high risk categories have concentrations of COCs that are sufficient to adversely affect the metabolic rate of microorganisms and, as a result, the activity of the microbial community. Because many of the COCs in the BI AOC are considered to be relatively persistent in surficial and deeper sediments and there is little evidence that COC concentrations are decreasing over time (see Table E1-7 of Appendix E1), it is likely that such effects will continue to impact the microbial community unless corrective action is taken to reduce risks in high (and possibly certain indeterminate) risk locations.

5.3 Integrated Assessment of Risks to the Microbial Community in the Middle Calcasieu River Area of Concern

A total of 163 sediment samples were collected within the MCR AOC to support an assessment of the risks posed to the microbial community associated with exposure to sediment-associated COPCs. The results of this assessment indicated that exposure to whole sediments in the MCR AOC generally posed a low risk to microorganisms (i.e., average of the final risk scores of 0.845; n=163). Seventy-three percent of the sediment samples (i.e., 119 of 163) from this AOC had low final risk scores (i.e., < 2; Table C-41). Nevertheless, indeterminate risks to the microbial community were indicated for 27% (44 of 163) of the sediment samples from the MCR AOC (Table C-41). Consistent with the results of the preliminary analysis of the areal extent of effects, the locations where contaminated sediments posed the highest risks (i.e., relative to the activity of the microbial community) included the western shoreline of Middle Calcasieu River mainstem from Bayou d'Inde to Moss Lake, portions of Prien Lake, portions of Moss Lake, Indian Wells Lagoon, and portions of the old river channel within the Middle Calcasieu River mainstem reach (Figures C-37a, C-37b, and C-37c).

The biological conditions that occur within the three risk categories indicate that sediments posing indeterminate and high risks are more toxic to the bacterium, *Vibrio fisheri*, than are sediments posing low risks (Table C-42). The mean EC_{50}-bioluminescence was 11.1$\pm$8.4 for the whole-sediment samples that were classified into the low risk category (n=84). By comparison, the mean EC_{50}-bioluminescence was 0.50$\pm$0.32 (n=5) for the sediment samples that were classified into the indeterminate category. No samples were classified into the high risk category, so it was not possible to determine the average EC_{50} for this risk category. Therefore, the sediment samples included in the indeterminate and high risk categories have concentrations of COCs that are sufficient to adversely affect the metabolic rate of microorganisms and, as a result, the activity of the microbial community. Because many of the COCs in the MCR AOC are considered to be relatively persistent in surficial and deeper sediments and there is little evidence that COC concentrations are decreasing over time (see Table E1-7 of Appendix E1), it is likely that such effects will continue to impact the microbial community unless corrective action is taken to reduce risks in high (and possibly certain indeterminate) risk locations.

6.0 Summary and Conclusions

The risks to microbial communities posed by exposure to whole sediments were assessed in the Calcasieu Estuary. In total, information on two lines of evidence was used to determine if the activity of the aquatic microbial community has been adversely affected or is likely to have been adversely affected by exposure to sediments in the estuary relative to reference conditions. The two lines of evidence that were considered in the assessment included whole-sediment chemistry and whole-sediment toxicity.

The results of this assessment indicated that exposure to whole sediments from the Calcasieu Estuary posed variable risks to microbial communities (i.e., risks were classified as low for 51% and indeterminate for 49% of the 624 sediment samples collected within the three AOCs investigated; Table C-41). Of the three AOCs considered, the risks to the microbial community were highest in Bayou d'Inde. Within this AOC, sediment samples from the lower portions of Upper Bayou d'Inde, Middle Bayou d'Inde, Lower Bayou d'Inde mainstem, and Lockport Marsh posed the highest risks to the microbial community (Figures C36a and C36b). Although risks to the microbial community were generally lower in the UCR AOC and MCR AOC, sediments posing indeterminate risks were identified in the northern portions of Clooney Island Loop, Clooney Island barge slip, the northern, central, and southern portions of Coon Island Loop, the western shoreline of Middle Calcasieu River mainstem from Bayou d'Inde to Moss Lake, Moss Lake, Prien Lake, Indian Wells Lagoon, and portions of the old river channel within the Middle Calcasieu River mainstem reach (Figures C-35a, C-35b, C-37a, C-37b, and C-37c). Risks to the microbial community are generally low throughout the reference areas (Figure C-38).

The results of the biological investigations conducted during the RI indicate that the magnitude of effects tends to increase with increasing risk to the microbial community. The average EC_{50}-bioluminescence was 11.1$\pm$8.4 % sediment wet weight/mL (n=84) for the whole-sediment samples that were classified into the low risk category (Table C-42). For the samples that were classified into the indeterminate risk category, a mean EC_{50}-bioluminescence of 0.5$\pm$0.3 % sediment wet weight/mL (n=5) was calculated. Together, these results demonstrate that the metabolism of microorganisms has been impaired in response to exposure to contaminated sediments at certain locations in the Calcasieu Estuary. An assessment of the risks that are posed to the microbial community by exposure to whole sediment in the Calcasieu Estuary (i.e., on a station-by-station basis) is presented in Tables C-43 to C-61.

The results of this assessment indicated that a number of substances are causing or substantially contributing to adverse effects on the microbial community in the Calcasieu Estuary (i.e., relative to reference conditions). More specifically, the COCs in the estuary were considered to include:

- PAHs (1,1-biphenyl, 2-methylnaphthalene, acenaphthene, acenaphthylene, anthracene, fluorene, naphthalene, phenanthrene, total LMW-PAHs, benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, fluoranthene, indeno(1,2,3-cd)pyrene, pyrene, total HMW-PAHs, and total PAHs);
- PCBs (total PCBs); and,
- Phthalates (BEHP).

All of these substances occurred in whole-sediment samples at concentrations in excess of those observed in samples from reference areas and in excess of the selected benchmarks. In addition, the concentrations in the effects distribution were generally higher than the concentrations in the no effects distribution for the selected measurement endpoint (i.e., bioluminescence of the bacterium, *Vibrio fisheri)*. Hence, the COCs identified above should be considered to be the highest priority for developing preliminary remediation goals.

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